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Original Article

Human cystic echinococcosis, trichinellosis, and toxocariasis in Bulgaria: an update of data for 2015-2017

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Abstract

Background: The purpose of our study is to perform a retrospective analysis of the prevalence of some zoonotic helminth infections (trichinellosis, cystic echinococcosis, and toxocariasis) for the period 2015-2017, given their important medical and social significance for the public health system in Bulgaria and other endemic countries.

Methods: We used data from the National Centre of Infectious and Parasitic Diseases and Regional Health Inspectorates for all registered cases of cystic echinococcosis and trichinellosis during the study period and data from the Department of Parasitology and Tropical Medicine on all cases seropositive for toxocariasis.

Results: For the study period in the country were reported 800 confirmed cases of hydatid disease of which 715 (89.4%) primary and 85 (10.6%) cases of relapse. The group of children and adolescents accounted for 21% of the total number of infected. Ten trichinellosis outbreaks were also recorded, involving the population of 16 settlements across 10 of the 28 districts of the country. Contaminated with *Trichinella* larvae meat was consumed by 293 persons of whom laboratory confirmed infection was found in 113 individuals (38.6%). For the period, 408 persons with clinical symptoms suspicious for toxocariasis were tested and 78 (19.1%) of them were positive for the presence of specific anti-*Toxocara* antibodies.

Conclusions: Parasitic zoonotic diseases have endemic spread in many countries around the world and pose a problem for public health. We present data on three helminth diseases, two of which (cystic echinococcosis and trichinellosis) are still widespread in Bulgaria and are of great medical and social importance.

Keywords: echinococcosis, toxocariasis, trichinellosis

Introduction

Several hundred infectious diseases are classified as zoonotic and are caused by bacteria, viruses, fungi, or parasites that can be transmitted from animals to humans [1]. The majority of human parasitic diseases due to helminths and protozoa are zoonoses [2]. Parasitic zoonoses are with cosmopolitan distribution and increasingly are recognized as a significant threat to public health both locally and globally [3]. Their widespread prevalence can be associated with a number of human factors including social and dietary habits

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as well as the increased mobility of the human population [4].

Cystic and alveolar echinococcosis caused by the cestode species *Echinococcus granulosus* and *Echinococcus multilocularis* account for high morbidity in many areas of the world including large parts of Europe, North and South America [5].

Cystic echinococcosis (CE) is a foodborne parasitic disease which may take a severe clinical course that often leads to prolonged disability. The disease tends to relapse frequently after treatment and sometimes can cause permanent disabilities and even death. For the period 1996 - 2014, a total of 9673 primary cases of CE were recorded in the country, with average morbidity of 6.25 per 100,000. By the number of cases and annual incidence Bulgaria ranks first among the member states of the European Union (EU) [6].

Available data suggest that all *Trichinella* taxa can infect humans, but *T. spiralis* and *T. britovi* are the most frequent species involved in human disease. Human trichinellosis is acquired through the consumption of insufficiently heat-treated meat from domestic or wild animals. The severity of the symptoms depends on the number of ingested *Trichinella* larvae and the immune response of the host [7]. The incubation period for the development of disease after the consumption of contaminated meat ranges from one to fifty-one days [8]. In recent years, Bulgaria has been among the EU member states with the highest incidence of trichinellosis among humans. According to European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) in 2016, Bulgaria had the highest notification rate in the EU (0.49 cases per 100,000), followed by Romania and Croatia with 0.13 and 0.12 cases per 100,000, respectively [6].

Human toxocariasis is a soil transmitted parasitic disease caused by dogs' and cats' nematodes *Toxocara canis* and *Toxocara cati*. Currently, it is one of the most commonly reported infections with a worldwide distribution [9]. In Bulgaria from 1997 to 2006 were studied 1447 serum samples from patients suspected of toxocariasis, collected from different parts of the country. Seroprevalence of 12.25% was found among them [10].

The purpose of our study is to conduct a retrospective analysis of the prevalence of some zoonotic helminth infections (trichinellosis, cystic echinococcosis, and toxocariasis) for the period 2015-2017, given their important medical and social significance for the public health systems in Bulgaria and in all regions where their transmission is endemic.

Methods

Study design

The study encompasses a period from January 2015 to December 2017 and was conducted at the Department of Parasitology and Tropical Medicine (DPTM) at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria. Aggregated data on all individuals in the country infected with *Trichinella* spp., *Echinococcus granulosus*, and *Toxocara* spp. were collected from the annual reports issued by the Regional Health Inspectorates (RHI) and from the own records of NCIPD on the performed through the period routine and confirmatory serological tests.

Patients

The study includes data of all confirmed cases of cystic echinococcosis and trichinellosis in Bulgaria, diseases that under national law are subject to mandatory notification. Also, we included DPTM data from the serologic tests for toxocariasis in subjects suspected of the visceral or ocular form of the disease.

Parasitological diagnosis

Diagnostic methods applied in cases of CE were fully consistent with the recommendations of the WHO [11]. Used were imaging techniques (ultrasound, computed axial tomography, magnetic resonance, radiography), immunological tests (commercial enzyme-linked immunosorbent assay (ELISA), and commercial Western blot, according to the manufacturer instructions), microscopic examination of hydatid fluid and methylene blue test and PCR methods for species identification of *E. granulosus*.

The parasitological diagnosis of trichinellosis and toxocariasis in humans is performed by immunological methods: commercial enzyme-linked immunosorbent assay, and commercial Western blot, according to the manufacturer instructions. Also, at NCIPD is carried out species identification of *Trichinella* larvae with biomolecular methods [12].

Data analysis

The data analysis focused on the incidence, age, and gender of patients. Mean, standard deviation, standard error, and confidence interval of mean (95%) were used. The statistically significant difference in age and gender distribution was analyzed by Mann-Whitney test. Values of $p < 0.05$ were considered statistically significant.

Ethical consideration

The present study is not part of a funded research project, and the data were collected by routine diagnostic activities and epidemiological investigations carried out under Bulgarian legislation. As the presented data are aggregated an ethical approval and consent to participate were not applicable.

Results

Cystic echinococcosis

For the studied by us period in Bulgaria were recorded 800 confirmed cases of CE, of which 715 (89.4%) primary and 85 (10.6%) cases of relapse. Table 1 presents the distribution of the cases by age and gender. Of all patients, 168 (21%) were children and adolescents. The most affected age groups were 25-29 years (N=67, 8.4%) and 30-34 years (N=74, 9.2%). By gender 51.5% of the patients were female and 48.5% male. Annual morbidity ranged from 4.35 in 2015 to 3.1 per 100,000 in 2017 (mean annual morbidity over the period, 3.56 per 100,000). During the period was recorded one case with a fatal outcome, a 34-year-old woman died from anaphylactic shock as a result of a spontaneously ruptured hydatid cyst in the liver. In terms of localization and number, a solitary cyst in the liver was found in 561 cases, solitary cyst in the lungs in 156 patients, in 13 the cyst was in the spleen, eight patients were with solitary cyst in the kidney, in two in the mesentery and in another 10 patients' solitary cysts were found in the myocardium, CNS, mammary gland, subcutaneous tissue, and ovary. Ten patients were with cysts affecting the lung and the liver and 40 patients were with multiple cysts localized in different parts of the body.

Table 1. Distribution of cystic echinococcosis by age groups and gender (2015-2017)

Age groups	Gender		Percent (%)	Mean	Std. Deviation	Lower 95% CI of mean	Upper 95% CI of mean
	Male	Female					
0-4	11	1	1.5	6.000	7.071	-57.53	69.53
5-9	32	21	6.6	26.50	7.778	-43.38	96.38
10-14	28	23	6.4	25.50	3.536	-6.265	57.27
15-19	30	22	6.5	26.00	5.657	-24.82	76.82
20-24	36	23	7.4	29.50	9.192	-53.09	112.1
25-29	31	36	8.4	33.50	3.536	1.735	65.27
30-34	40	34	9.2	37.00	4.243	-1.119	75.12
35-39	29	31	7.5	30.00	1.414	17.29	42.71
40-44	21	32	6.6	26.50	7.778	-43.38	96.38
45-49	25	26	6.4	25.50	0.7071	19.15	31.85
50-54	19	21	5.0	20.00	1.414	7.294	32.71
55-59	12	25	4.6	18.50	9.192	-64.09	101.1
60-64	22	36	7.2	29.00	9.899	-59.94	117.9
65-69	27	27	6.7	27.00	0.0	27.00	27.00
70-74	12	20	4.0	16.00	5.657	-34.82	66.82
75-79	10	18	3.5	14.00	5.657	-36.82	64.82
>80	5	14	2.4	9.500	6.364	-47.68	66.68
Total	390	410	100	400.0	14.14	272.9	527.1
Male vs. Female, Mann Whitney test: p-value = 0.7659							
Children vs. Adults, Mann Whitney test: p-value = 0.1000							

Trichinellosis

Ten outbreaks of trichinellosis were recorded in 16 settlements of 10 regions of the country. Contaminated with *Trichinella* larvae meat was consumed by a total of 293 persons, of whom laboratory confirmed infection was found in 113 patients (38.6%), (Table 2). The age groups from 4 to 74 years were affected, but the largest share was of the groups 35-39 (15%) and 40-44 years (16.8%). The proportion of infected children and adolescents was 13.3%, and by gender distribution, the number of infected males was higher (67.3%). The registered annual incidence from trichinellosis was 0.31 per 100,000 (2015), 0.5 (2016), and 0.78 (2017). In five outbreaks source of

infection was domestic pork, in two, meat from wild boar, and in three outbreaks the source remained unknown. In three trichinellosis outbreaks, the species of the causative agent was identified by biomolecular methods performed at the NRL of NCIPD. In one outbreak was isolated *T. spiralis* from the meat of domestic pig and in the other two outbreaks, *T. britovi* was isolated also from the meat of domestic pigs. In the other seven outbreaks, the *Trichinella* species was not established due to lack of material to be investigated. All infected persons with a laboratory-confirmed diagnosis were treated with albendazole in standard doses and regimens.

Table 2. Distribution of trichinellosis by age groups and gender (2015-2017)

Age groups	Gender		Percent (%)	Mean	Std. Deviation	Lower 95% CI of mean	Upper 95% CI of mean
	Male	Female					
0-4	1	0	0.9	0.5000	0.7071	-5.853	6.853
5-9	4	2	5.3	3.000	1.414	-9.706	15.71
10-14	5	1	5.3	3.000	2.828	-22.41	28.41
15-19	1	1	1.8	1.000	0.0	1.000	1.000
20-24	3	3	5.3	3.000	0.0	3.000	3.000
25-29	7	2	8	4.500	3.536	-27.27	36.27
30-34	9	3	10.6	6.000	4.243	-32.12	44.12
35-39	13	4	15	8.500	6.364	-48.68	65.68
40-44	13	6	16.8	9.500	4.950	-34.97	53.97
45-49	5	3	7.1	4.000	1.414	-8.706	16.71
50-54	9	2	9.7	5.500	4.950	-38.97	49.97
55-59	1	3	3.5	2.000	1.414	-10.71	14.71
60-64	2	3	4.4	2.500	0.7071	-3.853	8.853
65-69	2	3	4.4	2.500	0.7071	-3.853	8.853
70-74	1	1	1.8	1.000	0.0	1.000	1.000
75-79	0	0	0	0.0	0.0	0.0	0.0
>80	0	0	0	0.0	0.0	0.0	0.0
Total	76	37	100	56.50	27.58	-191.3	304.3
Male vs. Female, Mann Whitney test: p-value > 0.9999							
Children vs. Adults, Mann Whitney test: p-value = 0.1000							

Toxocariasis

In Bulgaria laboratory tests for toxocariasis are carried out mainly in the NRL at the NCIPD. For the study period, a total of 408 people with the suspected disease were examined. Most of them were individuals with peripheral eosinophilia, allergic symptoms, or granulomatous formations in the eyes. Of all examined persons 78 (19.1%) were positive for the presence of anti-*Toxocara* antibodies. Gender and age distribution are presented in Table 3. The relative share of children and adolescents is 17.9% (N=14), and the seroprevalence among males is higher (N = 46, 59%) than in females (N=32, 41%).

Discussion

Parasitic zoonoses are diseases posing a major challenge to public health systems in endemic countries. For Bulgaria, of great medical and social significance are the cystic echinococcosis and trichinellosis. For the studied by us three-year-period the average incidence was 3.56 per 100,000. Comparing the data from our previous study covering the period 1996-2013 with an established mean incidence of 6.7 per 100 000 [13], it can be ascertained that there is a nearly twofold reduction in human morbidity. This is also confirmed by the European Union Synthesis Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Related Epidemic Outbreaks for 2016, according to which cases from Bulgaria decreased by 30.3% from 2008 to 2016 [6]. We consider that the decreasing annual incidence of CE in the country is a result of the recently implemented National Program for Control of Cystic Echinococcosis in Humans and Animals (2004-2008). Nevertheless, the incidence of CE in Bulgaria is much higher than in other EU member states. Whereas in 2015 the total annual incidence in the EU Member States is 0.2 per 100,000, the incidence in Bulgaria is 4.35 per 100,000 [14]. Similar is the situation in 2016 with a recorded incidence in the EU of 0.19 per 100,000 and 3.23 per 100,000 for Bulgaria [6].

Table 3. Seroprevalence of anti-*Toxocara* IgG antibodies by age groups and gender (2015-2017)

Age groups	Gender		Percent (%)	Mean	Std. Deviation	Lower 95% CI of mean	Upper 95% CI of mean
	Male	Female					
0-4	5	2	9	3.500	2.121	-15.56	22.56
5-9	4	1	6.4	2.500	2.121	-16.56	21.56
10-14	0	0	0	0.0	0.0	0.0	0.0
15-19	2	0	2.6	1.000	1.414	-11.71	13.71
20-24	2	0	2.6	1.000	1.414	-11.71	13.71
25-29	2	1	3.8	1.500	0.7071	-4.853	7.853
30-34	4	4	10.3	4.000	0.0	4.000	4.000
35-39	1	3	5.1	2.000	1.414	-10.71	14.71
40-44	10	3	16.7	6.500	4.950	-37.97	50.97
45-49	7	0	9	3.500	4.950	-40.97	47.97
50-54	2	2	5.1	2.000	0.0	2.000	2.000
55-59	3	5	10.3	4.000	1.414	-8.706	16.71
60-64	1	4	6.4	2.500	2.121	-16.56	21.56
65-69	2	5	8.9	3.500	2.121	-15.56	22.56
70-74	1	0	1.3	0.5000	0.7071	-5.853	6.853
75-79	0	2	2.6	1.000	1.414	-11.71	13.71
>80	0	0	0	0.0	0.0	0.0	0.0
Total	46	32	100	39.00	9.899	-49.94	127.9
Male vs. Female, Mann Whitney test: p-value = 0.4302							
Children vs. Adults, Mann Whitney test: p-value = 0.1000							

Of great concern is the high relative share of infected children and adolescents: 21% for the period 2015-2017, and 21.4% for 1996-2013, because this is an indicator of high disease transmission rates [13]. On one side, the possible reasons are the insufficient hygienic habits of the children and their frequent contact with dogs for play. On the other, the high incidence of CE in the country is due to a large number of dogs (wandering, yard, sheep) that are not regularly dewormed, particularly in areas with developed livestock breeding and especially sheep breeding [15]. The highest average incidence rate of CE was recorded in three regions: Sliven (14.11 per 100,000), Kardzhali (13.4 per 100,000), and Dobrich (9.24 per 100,000). In all three areas, sheep production is well developed. The territory of Sliven and its region falls within South-East Bulgaria, where 21.5% of sheep are grown in the country, Kardzhali is within the South-Central region, where 23.2% of the sheep are grown, and Dobrich is located within the North-Eastern region of the country, where 14.9% of the sheep are bred [16]. It is clear that the areas with the highest incidence for our survey period are located in territories where about 60% of sheep in the country are grown. It is estimated that in 2016, 4.36% of the sheep in Bulgaria are infected with *E. granulosus* [6]. Another interesting fact is that these areas are also characterized by ethnic heterogeneity of the population in them. In all of them, live compact masses of Roma origin. In our view, the cultural and social peculiarities of these groups have a significant impact on the prevalence of the disease among people. This view is supported by the fact that in the Northwest and Southwest regions of the country where the relative share of sheep breeding is over 30%, the incidence of CE is significantly lower - 2.57 per 100,000 and 3.02 per 100,000 [16]. The prevalence and the intensity of the parasitic infections are important indicators for the level of the epizootic process. In countries where these indicators are low (Germany, Sweden, Norway), echinococcosis among farm animals and humans is rare. The epizootiological and epidemiological significance of the dogs as a source of echinococcosis is determined by their purpose. The shepherd dogs are the most important as final hosts, because of the relatively high frequency of infection and their constant and close contact with the sheep [15]. There is no single register for the number of dogs in Bulgaria. Data from annual dog enumeration display a decrease in the number of wandering dogs in the country from 31,435 in 2011 to 26,890 in 2017 [17].

Regarding the clinical manifestation and organ localization of the hydatid cysts, our study has the following data: 93.75% (N =750) of patients had a single cystic lesion located in a single organ. The liver was affected in 70.13% of cases. The second common place for cyst localization was the lung, 19.5%, followed by the spleen, 1.62%. With the involvement of both, lung, and liver were 1.25% of the

patients, and 5% (N=40) were with multiple cysts localized in different parts of the body. In this respect, our data are similar to those of other authors [18-20]. The relative proportion of recrudescence was 10.62% (N=85), and our findings are similar to those of Velasco-Tirado et al., according to which the relative share of relapses after invasive treatment procedures is 11% in Spain [21], and Prousalidis, et al. who observed relapses in 8.7% of the patients with CE in Greece [22]. In Bulgaria, all persons treated with surgery or PAIR technique (Puncture, Aspiration, Injection, and Re-aspiration) are assigned with relapse preventing prophylaxis with albendazole, the duration is determined individually in each patient, depending on the clinical manifestation of the disease. Nevertheless, the number of relapses is relatively high. Regrettably, in the three-year period of our study, there was a case with a fatal outcome by anaphylactic shock, a consequence of a spontaneously ruptured hydatid cyst in the liver.

Echinococcosis is a serious social problem with many dimensions: human, medical, veterinary, economic, and environmental. We believe that from the side of human medicine, there are no particular difficulties with the diagnosis, treatment, and surveillance of the disease. Measures to reduce the incidence include enforcement of the preventive actions by the public health authorities with media collaboration and involvement.

Trichinellosis is the second most important parasitic zoonosis in Bulgaria. Outbreaks of human trichinellosis are recorded annually in the country [23]. Through the period of our study were recorded ten outbreaks with 113 infected persons, of which 86 had clinical symptoms and 27 were with an asymptomatic form of the disease. The food-specific attack rate we established for each year is 9.64% (2015), 36.92% (2016), and 37.24% (2017). When determining this indicator, we included only cases with clinically manifested illness. Nearly all age groups were affected (Table 2), and the proportion of infected adults was significantly higher (86.73%) than that of infected children and adolescents (13.27%). The age of infected patients varied from 4 to 74 years, with the largest number infected in the 30-44 age group (N=48, 42.48%). The gender distribution displays that majority of the patients were males (N=76, 67.26%) than females (N=37, 32.74%) but without statistically significant difference between them (Mann Whitney test: $p=0.9356$). The annual incidence displayed a trend to rise for our study period: from 0.31 per 100,000 (2015) to 0.79 per 100,000 (2017). By this indicator, Bulgaria also holds a leading position among the EU member states, where the average annual incidence in 2015 was 0.03 per 100,000 and 0.05 in 2016 [6,14]. Meat from domestic pigs was a source for infection in five of the outbreaks, wild boar meat was a source in two outbreaks and in tree source was not identified. In our opinion based on our previous studies [23] and the present one, a prerequisite for occurrence of outbreaks and cases of trichinellosis among the population in Bulgaria is the widespread in rural areas practice to breed domestic animals at home and to slaughter them in the back yards without provision of veterinary sanitary control over the harvested meat. The lack of stringent sanctions against violators of the meat production and poachers of wild animals is also important. Despite the systematically conducted educational work by human and veterinary doctors on trichinellosis among the population, the knowledge about the disease and its prevention is still far from a satisfactory level.

Toxocariasis is a parasitic zoonotic disease with various clinical manifestations, protracted course, and common recurrences. In Bulgaria the exact incidence of human toxocariasis still not established, but significant information has been gathered over the last 10 years [24]. For 2013-2014, by ELISA method for anti-*Toxocara* antibodies were tested 466 persons. Most of them were with clinical symptoms. A positive result was found in 90 of them (19.3%) [25]. Data obtained by the present study are similar. The proportion of positive patients is 19.1%, and most of them are males (59%). The relatively high number of seropositive individuals could be explained by the fact that subjects of the study were not randomly selected, but were patients with different clinical symptoms that could be associated with pathology caused by *Toxocara* larvae. Despite numerous literature data that younger people are a more vulnerable group in terms of toxocariasis [26], in our study the relative share of seropositive adults was almost equal to this of seropositive children and adolescents. Out of 78 children and adolescents, seropositive was 17.95%, whereas out of the 371 adults, 17.4% were with the presence of anti-*Toxocara* antibodies, and there is no statistically significant difference between the two groups (Mann Whitney test: $p\text{-value}=0.1000$). In contrast to age distribution, our data is similar to the literature regarding seroprevalence between urban and rural residents. Of the 363 surveyed urban residents presence of antibodies was found in 17.35% (N=63), while among 45 residents of rural areas the established seroprevalence was 33.33% (N=15). In Bulgaria, however, this division is quite conditional, because many urban residents have close links with rural areas (possessions and relatives) and frequently visit them on weekends and vacations [27].

The parasitological diagnosis of human toxocariasis is hampered by the fact that the parasite does not reach sexual maturity in the accidental human hosts, their eggs are not laid in the feces and microscopic methods cannot be used. For this reason, immunological methods (along with clinical evaluation) are leading in the diagnosis of the disease, mainly using ELISA to prove specific Ig G antibodies and Western blot [9]. When interpreting the results of serological tests, it should be borne in mind that the establishment of a positive result in a serological survey has limited diagnostic significance. Only patients with clinical signs compatible with toxocar iasis are appropriate for treatment due to the fact that with the available immunodiagnostic tests it is not possible to distinguish between recent and latent infection. Therefore, the serological test should be accompanied by the assessment of the level of eosinophilia and, if possible, by

determining the total serum IgE. All patients with clinical symptoms of toxocariasis and positive results in ELISA and Western blot have been treated with albendazole in classic doses and regimens in combination with antihistamines and/or corticosteroids.

Zoonotic diseases in general and parasitic ones, in particular, have endemic spread in many countries around the world and pose a challenge for public health. We present data on three helminth diseases, two of which (echinococcosis and trichinellosis) are still widespread in Bulgaria and are of great medical and social importance. Human toxocariasis has not been sufficiently studied in terms of epidemiology and clinical manifestations among the population of the country. All three diseases are completely preventable and their spread among people is due to bad health habits and insufficient veterinary and medical control over the sources of the disease. The major problem is the presence of a still large population of wandering dogs in the settlements, as well as courtyard, hunting and shepherd dogs, which are not regularly dewormed by their owners. While in legal hunting the game meat is a subject to veterinary control, such control usually is lacking in illegal hunting and in the domestic meat production. In this respect, the emphasis of the preventing measures should be on the implementation of educational programs and active screening of the population.

Conflict of interest

All authors declare that they have no conflict of interest.

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