THE PREVALENCE OF INTESTINAL PARASITES IN CENTRAL SLOVAKIA FROM THE YEAR 2000 – RETROSPECTIVE STUDY

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Abstract:

Introduction: The main group of helminthic infections in Europe includes soil-transmitted helminthiases as well as alimentary helminthiases and cestodes. Typical finds of coprological analyses in our climatic conditions include intestinal nematodes, tapeworms and intestinal protozoa.

Objectives: The primary objective of our study was to analyse changes in the prevalence of intestinal parasites in Slovakia.

Methods: The authors studied the prevalence of intestinal parasites in coprological samples taken from patients in Central Slovakia. Stool samples were prepared and analysed using an optical microscope with 160 – 400 x magnification.

Results: The most prevalent types of intestinal protozoa were *Giardia intestinalis* and *Entamoeba coli*. The most widespread species of helminths was *Enterobius vermicularis*. Since 2006, the prevalence of intestinal parasites has rapidly dropped in comparison to the 90s (0.44 % versus 1.32 % of positive findings, respectively).

Conclusion: The results show values rapidly decreasing towards zero for the prevalence of geohelminths and a significant decrease in intestinal protozoa. The authors propose possible epidemiological reasons for this sharp decrease, which points to the potential near elimination of intestinal parasites as a result of improvement in water management, waste management, personal hygiene, maintenance of public spaces as sandpits and parks.

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Keywords: geohelminths, intestinal protozoa, intestinal parasites, prevalence

Introduction

The main group of helminthic infections in Europe includes soil-transmitted helminthiases as well as alimentary helminthiases and cestodes. Typical finds of coprological analyses in our climatic conditions include intestinal nematodes such as *Ascaris lumbricoides*, *Trichuris trichiura* and *Enterobius vermicularis*; tapeworms such as *Taenia saginata, Taenia solium* and *Hymenolepis nana*; intestinal protozoa such as *Giardia intestinalis*, *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii* and *Entamoeba histolytica* in pre-cystic (minuta) form.

Intestinal nematodes

Ascariasis and trichuriasis belong to the most common diseases caused by geohelminths. These worms are characterised by direct development without intermediate hosts. The female's eggs do not become infectious immediately but need to mature in the ground. Larvae develop gradually from the first to the third - invasive stage. Egg maturation periods vary according to the type of worm, the temperature, moisture and oxygen levels. The optimum temperature of the external environment for *Ascaris lumbricoides* is 24–30°C; at this temperature, a larva will emerge from an egg in 15 days.

The *Trichuris trichiura* egg will develop in the external environment at a temperature of 15–40°C; in the optimal range 25–37°C, larvae mature in the egg in 25 days. Geohelminth eggs can remain infectious in the soil for up to five years. In open cesspits, the eggs will last 6 months and in closed pits for up to a year (Novakova, 2006, Ojha et al 2014). One of the most significant locations for the epidemiology of geohelminthiasis is reservoirs that receive communal wastewater contaminated with worms' eggs. The larvae can survive for a very long time in lakes and ponds. It is possible to become infected with geohelminthiasis by bathing, by consuming raw vegetables that have been contaminated by being irrigated with wastewater or fertilised with material taken directly from a cesspit or dung heap. Bigger particles such as helminth eggs are transported by flies on their external surfaces, i.e., exoskeletons (Graczyk, 2005).

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Enterobiasis (pinworm infection) is the only helminthiasis in which the eggs are transmitted directly from person to person. The formation of the larva begins in the female but for further development, the larvae require oxygen. To reach the open air, the female crawls out of the intestine and lays eggs in the perianal region of the host. At a temperature of 36–37°C, the larvae mature in 6–7 hours. This means that eggs laid in the evening have matured and are capable of invasion in the morning and hosts can be self-infected by eggs that they carry (Rawla, Sharma, 2018).

Tapeworms

Taenia saginata is a biohelminth which develops using intermediate hosts. It is found where beef is consumed without being properly cooked (Bogitsh, 2013). *Taenia solium* is a zoonotic parasite which infects humans and pigs resulting in the cysticercosis/taeniasis duo of neglected tropical diseases. It is commonly known as the pork tapeworm (O'Neal et al, 2014).

Hymenolepis nana differs from other tapeworms by not needing an intermediate host, and self-infection is possible, especially in children. Minor infections with a few small tapeworms do not cause problems and remain undetected. This is one reason for their low prevalence (Baron, 1996). Among children, fecal-oral (finger) transmission is possible. Cysts do not tolerate drying out and cannot survive in the external environment more than 10 days (CDC report Hymenolepiasis, 2017). It is possible for cysts to be transferred passively to food or drinks via insects. Cysts can survive in the intestines of a fly for 30 or more hours (Novakova, 2006). Small cystic stages of human-infectious intestinal protozoa can be ingested as well as transported on the exoskeleton (Graczyk, 2005).

Intestinal protozoa

Giardia lamblia (syn. *G. duodenalis*, *G. intestinalis*) cause giardiasis, amebiasis, the most common parasitic infection of the human intestine worldwide (Cama, Mathison, 2015). Infections caused by intestinal protozoa are transmitted mainly by water and foodstuffs (fruit, vegetables) contaminated by cysts. *G.lamblia* has high prevalence in companion animals (Esch, 2013). This parasite produces cysts, which are immediately infectious and can remain infectious in cool and moist environment for a long period (Cama, Mathison, 2015). In contrast to the year 2016 the distribution of confirmed giardiasis cases in Slovakia was 284. In the same year in the European Union were reported 18 985 cases of the confirmed giardiasis (ECDC, 2018).

Material and methods

Objective of our study is to analyse changes in the prevalence of intestinal parasites in Central Slovakia. Stool samples with a volume of 2 – 3 ml were taken using sampling and transport kits. Stool samples were prepared using the following techniques: the Kato technique and the Merthiolate-Iodine-Formaldehyde concentration method (M.I.F.C). The prepared samples were analysed using an optical microscope with 160 – 400 x magnification. The results were evaluated by one and the same parasitologist. The M.I.F.C. method was chosen as the most suitable for routine use because it is able to capture helminth eggs and protozoa cysts at the same time. While the thick smear is not suitable for diagnosing cysts, it can capture eggs with a higher unit weight, which might not be captured by the M.I.F.C. method. The combination of the two methods achieved a high capture rate both in terms of the spectrum of intestinal parasites diagnosed and in terms of the high probability of capture for each type of parasite. Tests for enterobiasis were conducted using the Graham-Brumpt method as modified by Palicka and Zitek, which is the most highly recommended technique for the detection of this infection (Novakova, 2006).

Results

Eleven different species of parasites (include protozoa and helminthes) was confirmed as an infectious agent in the study during years 2000-2006 (Table 1). Parasites ranked among the most prevalent parasite were *Giardia intestinalis*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Taenia saginata* and *Entamoeba coli* (Figure 1). During the years 2007-2018 the number of parasitic infections varied. In the year 2010 the number of cases of *E.vermicularis* increased to 207 cases, but since this year the number of infection caused by this pathogen has decreased. The highest number of infections by *G.intestinalis* occurred in the year 2012 with 159 cases during this period (Figure 2).

Table 1 shows the statistics on the coprological analysis of laboratory test for intestinal parasites with positive results in the early years covered by this research.

Table 1: Positive findings of each type of parasites during years 2000-2006

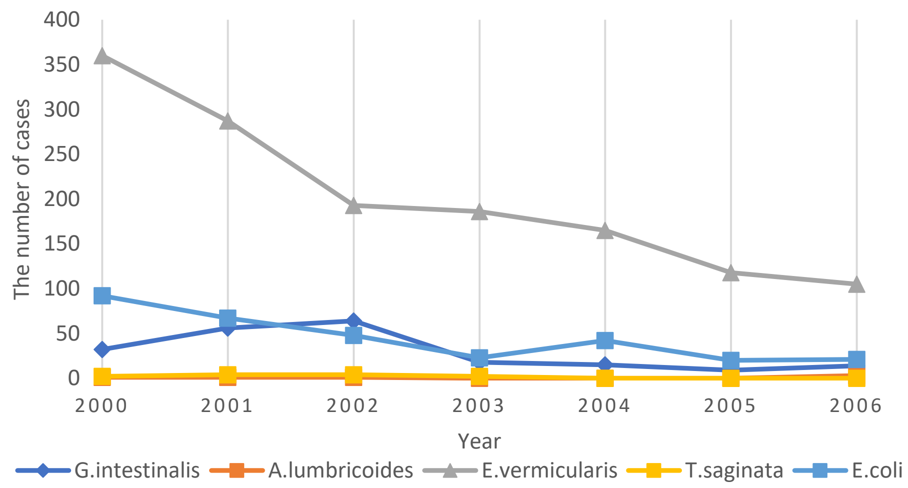
Year	Positive findings of each type – absolute number							
	G.i.	**E.c.**	**E.n.**	**E.hi.**	**A.l.**	**T.t.**	**E.v.**	**T.s.**
2000	32	92	0	0	1	3	360	2
2001	56	67	0	0	1	1	287	4
2002	64	48	1	0	1	0	193	2
2003	18	23	0	0	0	0	186	0
2004	15	42	0	0	0	0	165	0
2005	9	20	0	0	0	1	118	0
2006	14	21	0	0	3	0	105	1

G.i. – *Giardia intestinalis*, E.c. - *Entamoeba coli*, E.n. – *Endolimax nana*, E.hi. – *Entamoeba histolytica*– form minuta, A.l. – *Ascaris lumbricoides*, T.t. – *Trichuris trichiura*, E.v. – *Enterobius vermicularis*, T.s. – *Taeniarhynchus(Taenia) saginata*, H.n. – *Hymenolepis nana*, S. sp. – *Strongyloides* species

Source: Clinical Biochemistry (2000-2006)

Figure 1 shows number of the most prevalent types of parasites during years 2000-2006

Figure 1: The most common parasites during the period of years 2000-2006

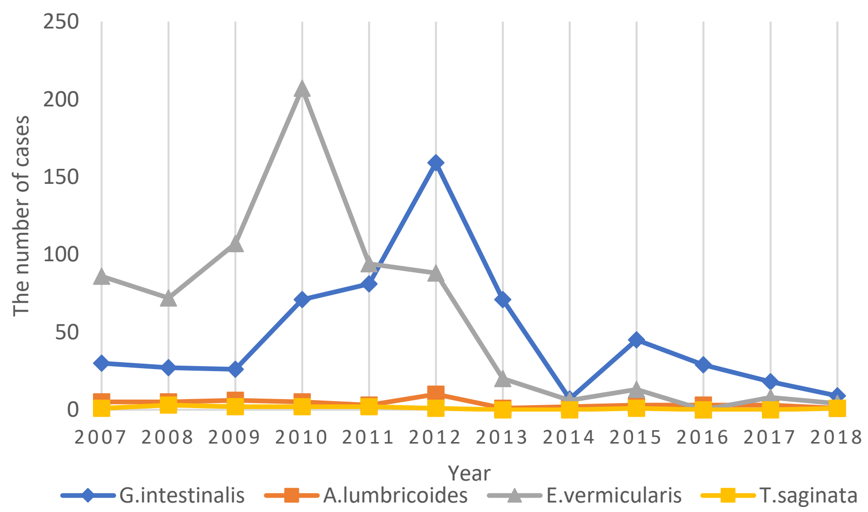


Source: Clinical Biochemistry (2000-2006)

The prevalence of chosen parasite had a decreasing trend among these years.

Figure 2 shows the most common human parasites through the years 2007-2018

Figure 2: The most common parasites during the period of the years 2007-2018



Source: The Regional Public Health Authority (2007-2018)

Among years 2007-2018 parasitic infections varied according the graph, but all infections show decreasing trends actually. Data about the number of parasitic infections caused by *E.coli* through the years 2007-2018 had not been available.

The figures clearly show values rapidly decreasing towards zero for the prevalence of geohelminths and a significant decrease in intestinal protozoa. Since 2003 the prevalence of intestinal parasites has been at level around 0.5%, with geohelminths representing just hundredths of a percent. Our findings correlate with findings of decreased prevalence of intestinal parasites in other regions of central Slovakia – Turiec and Banska Bystrica (Straka, 2001). Although enterobiasis has declined, it continues to have relatively high prevalence. Diagnosis of enterobiasis in the laboratory is affected by many special factors related to the parasite's biological cycle and its method of transmission. The collection technique must be adapted to the fact that pinworms are not found in stools (except in massive infections) but in the perianal folds. There are several obstacles to implementing the Graham method in the routine work of children's general practitioners and there are also frequent technical faults and false negatives. We therefore suspect that the real rate of prevalence of enterobiasis could be higher than the table indicates.

Discussion

It is evident that the prevalence of intestinal parasites is indirectly proportionate to the application of relevant hygienic measures (Ojha, 2014). The higher the level of hygiene in the population, the lower the prevalence of intestinal parasites. On the other hand, the prevalence of infections such as giardiasis will be high in areas where people draw water from their own sources (wells) or store it in unprotected vessels (uncovered jugs, barrels, cisterns). Underground reservoirs are exposed to the risk of faecal contamination and endemic giardiasis contamination. There is a high risk of contamination in agricultural areas where wastewater is used for irrigation. The epidemiological factors responsible for the sharp fall towards zero in intestinal parasites may include the adoption of Act No 364/2004 on water and amending Act No 372/1990 on offences, as amended (the Water Act) and Act No 442/2002 on public water supplies and public sewerage systems:

1. cancellation of private drinking water sources (wells) and connections to public water supply systems (88.6% of the population receive drinking water from public water mains);
2. communal wastewater must be disposed of in a public sewerage system.

Another significant contribution to the decrease in the prevalence of intestinal parasites has come from ecological changes:

1. upkeep of public space (sandpits, parks);
2. changes on private land (cancellation of dry WCs, cesspits and dung heaps, planting of decorative plants rather than vegetables in gardens);
3. individual prophylactic measures (washing hands, use of gloves when working in gardens, washing fruit and vegetables, etc.).

In the year 2007 European Commission approved The Operational Programme Environment for Slovakia. The percentage of the population connected to wastewater treatment plants after this programme should increase to 81% and the population supplied with drinking water from public water supply network should increase to 91%. The specific objectives of the Operational Programme Environment 2007-2013 were the construction of water supply and sewerage infrastructure (Huska, 2007). According Water Research Institute in Slovakia the level of connection of the inhabitants to the public sewage system was 58.2 % in 2007. After 10 years the level of connection reached 67.7% in 2017 (Korenova, 2019). The incidence of intestinal parasite infection can be reduced by improved infrastructure for drinking water and sewage system (Ostan et al., 2007). The accurate identification of parasites in stool specimen is determined by appropriate and sensitive techniques. Some methods require high level of skills for optimal interpretation (McHardy et al., 2013). Our study is based on traditional microscopic evaluation of the biological samples. The microscopy remains the gold standard of diagnostic methods in many diagnostic laboratories. However, in recent years, research has been focused on the development of serological, molecular and proteomic methods to improve the diagnosis of parasitic diseases. Serological methods for diagnosis intestinal parasites include direct or indirect-fluorescent antibody, indirect hemagglutination, immunoblott, ELISA and imunochromatography

(Ndao, 2009; Ricciardi, Ndao, 2015). A direct immunofluorescence assay is used for diagnosis *Giardia duodenalis* (CDC report - Giardiasis, 2013). The serological method such as ELISA is applied in many reference laboratories, it´s advantage is that it is the lowest costing method for health insurance. On the other hand, the spectrum of detectable pathogens is small (Kompanikova et al., 2017). All molecular approaches use for diagnosis intestinal parasites incorporate methods like loop-mediated isothermal amplification, multiplex PCR and real-time PCR. Helminthes can be diagnosis by proteomic approach, mass spectrometry (Ndao, 2009; Ricciardi, Ndao, 2015). Mass spectrometry such as MALDI-TOF (Matrix-assisted laser desorption ionization – time-of-flight) is a precise method, which identify microorganisms by the comparison of the protein content with reference range of proteins in the database (Neuschlova et al., 2017). This method is poorly used in diagnostic parasitology, but according Villegas et al. (2006) this method can be applied for diagnostic intact cyst of *Giardia lamblia* or *Giardia muris*. Many new technologies in diagnostic laboratories represent challenges, but they can help to understand public health problems caused by parasitic disease (Singh et al., 2009).

Conclusion

The elimination of intestinal parasites in central Slovakia in the near future remains questionable. In Eastern Europe, the soil-transmitted helminth infections, giardiasis, and toxoplasmosis remain endemic (Hotez, 2011). The main task of the Epidemiological Information System (EPIS) of the Public Health Authority in the Slovak Republic is to monitor the epidemiological situation of human infectious diseases in Slovakia. The system does not contain the official information about the prevalence of endoparasitic diseases with the exception of toxoplasmosis and taeniosis (Dudlova et al., 2016). There is a lack of epidemiological data that can be applied for testing guidance for the most prevalent health complications in the refugee population at present (Mockenhaupt et al., 2016). The precise, fast and standardized diagnostic methods in parasitology are necessary for the correct analysis of the epidemiological situation.

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