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Toxocariasis in urban environment of western Slovakia

F. ONDRISKA[1], K. MAČUHOVÁ[2], J. MELICHEROVÁ[2], K. REITEROVÁ[3], D. VALENTOVÁ[4], V. BELADIČOVÁ[4], J. HALGOŠ[2]

[1]HPL (Ltd) Medical Laboratories, Istrijská 20, 841 07 Bratislava, Slovak Republic, E-mail: *ondriska@hpl.sk*; [2]Department of Ecology, Faculty of Natural Sciences Comenius university, Mlynská dolina 842 15, Bratislava, Slovak Republic, E-mail: *katarina.macuhova@gmail.com*, *halgos@fns.uniba.sk*; [3]Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic, E-mail: *reiter@saske.sk*; [4]State veterinary and Food Institute, Botanická ulica 15, 842 52 Bratislava, Slovak Republic, E-mail: *valentova@svuba.sk*, *beladicova@svuba.sk*

Summary

The aim of our study was to determine the prevalence of toxocariasis in Bratislava and smaller towns in western Slovakia. During 2006 – 2011, sand samples collected from 121 sandpits were investigated: 63 sandpits were from Bratislava City and 58 from sandpits in towns outside Bratislava (Malacky, Pezinok Stupava). In Bratislava, 27 % of examined sandpits were contaminated with *Toxocara* spp. eggs. In smaller towns eggs of *Toxocara* spp. were found from three sandpits (6.8 %) of Pezinok and Stupava only. In Malacky, no faeces and no eggs were found in any sandpits. Faecal samples of 1436 dogs and 263 cats were investigated. *T. canis* eggs were found in the faeces of 16.5 % dogs and *T. cati* in 18.6 % of examined cats. Toxocariasis of dogs was significantly higher in smaller towns such as the city Bratislava ($\chi 2 = 10.88$ for $P \leq 0.001$). The difference in prevalence of *T. cati* in cats bred in Bratislava and outside Bratislava was not confirmed ($P \leq 0.05$). 382 pregnant women were examined by ELISA. Anti-*Toxocara* antibodies were detected in 32 women (8.4 %). The difference in seroprevalence of women coming from Bratislava (6.6 %) and smaller towns outside Bratislava (11.0 %) was not statistically significant ($\chi 2 = 1.6$; $P \leq 0.05$).

Keywords: *Toxocara canis*; *Toxocara cati;* toxocariasis; sandpits; prevalence

Introduction

Human larval toxocariasis ranks among the most serious zoonotic infections worldwide, being the most commonly diagnosed tissue helminthosis in Slovakia. The causal agents are the larvae of *Toxocara canis* (dog roundworm) and *Toxocara cati* (feline roundworm) migrating through the tissues. Humans become infected by ingesting of viable embryonated eggs, shed via the faeces of infected animals in the large quantities into the environment. The human infection occurs most often in children; usually those with geophagia, as well as due to inadequate hygiene, by licking contaminated fingers, toys and other objects (Eberhard, 1998; Kinčeková *et al*., 1999; Reiterová *et al*., 2003; Alderete *et al*., 2003). An important mode of transmission is consumption of raw unwashed food, especially vegetables. The risk of infection is higher in professions involving a close contact with soil, wastewaters, and exposure to dog and cat faeces (Deutz *et al*., 2005). The infection can be transmitted also by contaminated passive vectors (synantropic flies) as well as by consumption of undercooked meat or viscera from a paratenic host (Pegg, 1971; Morimatsu *et al*., 2006). The possibility of congenital infection in humans is being discussed (Reiterová *et al*., 2001). In humans, the *Toxocara* spp. larvae never mature into adult worms, but the ascarid larvae of *Toxoca-ra canis* and *T. cati* can penetrate into various organs, whe-re they may survive for many years. Their migration may cause the mechanical damage of the tissues and formation of multiple eosinophilic granulomas sized 1 – 2 mm. They are localised predominantly in the liver, lungs, in the heart muscle, kidneys, intestinal wall, mesenteric nodes, pancreas and brain (Mirdha & Khokar, 2002). The clinical signs or symptoms of *Toxocara* infection include abdominal pain, headaches, anorexia, lymphadenitis, symptoms of atopic disease (Wiśniewska-Ligier *et al*., 2012). One of the most serious complications is the devastating ocular effect (Pivetti-Pezzi, 2009; Veralo *et al*., 2012).

The contamination of urban and rural environment by *Toxocara* eggs and larval toxocariasis in humans and animals are a common problem not only in Slovakia, but worldwide. *Toxocara* spp. eggs can be commonly found in soil, sand and similar loose substrates, which animals very much like to defecate on. Thus are contaminated faeces spread into the environment, even over great distances (Uhlíková & Hübner, 1983; Reiterová *et al*., 2003). Seriously contaminated sites are public parks, where eggs

are being dispersed into the air during mowing the lawn (Coelho, 2004; Totková *et al*., 2006). The number of dogs and cats, especially in urban areas, is ever so increasing, so their share on environmental contamination with *Toxocara* eggs has often been discussed. But stray cats still play an important role in epidemiology of toxocariasis (Talvik *et al*., 2006).

The aim of our study was to determine the prevalence of toxocariasis in Bratislava and smaller towns in western Slovakia. The project consisted of three partial tasks: 1. Detection of environmental contamination (sandpits, parking areas) by *Toxocara* spp. eggs in selected districts of Bratislava City: Devínska Nová Ves, Lamač, and Old Town, and in the small towns in western Slovakia (Malacky, Stupava, Pezinok). 2. Identification of *Toxocara canis* and *Toxocara cati* in dogs and cats kept in Bratislava and three cities in western Slovakia. 3. Determination of seroprevalence of larval toxocariasis in selected samples of healthy human population in Bratislava and three cities in western Slovakia.

Materials and methods

Biological material

During 2006 – 2011, sand samples collected from 121 children sandpits in order to identify the incidence of *Toxocara* spp. eggs in the environment were investigated. Out of them 63 sandpits were from Bratislava city (Devínska Nová Ves – 33, Lamač – 19, Old Town – 11), and 58 sandpits were located in towns outside Bratislava (Malacky – 23, Pezinok – 21, Stupava – 14). Faeces present in the sandpits were also collected. The whole material included dog faeces; cat faeces were not found.

The prevalence of toxocariasis in dogs and cats was determined in the samples of faeces from 1436 dogs (621 from Bratislava and 815 places outside Bratislava) and 263 cats (114 from Bratislava and 149 from towns outside Bratislava). The study included dogs and cats kept in households that have a close contact with the external environment.

To determine the seroprevalence of toxocariasis in a healthy human population, sera of pregnant women examined within preventive serological screening of toxoplasmosis were used. Altogether 382 sera of *Toxoplasma*-negative pregnant women aged from 16 to 44 years were examined; out of them 228 samples were from Bratislava and 154 sera were from Malacky, Stupava, and Pezinok. Clinical symptoms of the disease were not observed in any of the females. The samples were collected at the health care centres within the catchment area of the HPL (Ltd) Medical Laboratories in Bratislava.

The sand samples were examined according to the method of Červová (1986). Out of each square meter of sand, a representative sample of approximately 50 g of sand was examined. It was achieved by mixing sand from 20 samples (approximately 1000 g of sand) collected at various depths (up to 30 cm). The samples were stored in a cool box (4 ˚C) until the examination and were processed by the concentration method using Tween 40 and $NaNO_3$. After the flotation, the parasite eggs were microscopically examined with a 100, 200, and 400x magnification.

The faeces collected from sandboxes were examined microscopically, after concentration with flotation method with zinc sulphate ($ZnSO_4$) (Tolarová *et al.*, 1986). Similarly were examined the samples of cat and dog faeces, sent from veterinary practices and health centres to the

Table 1. The incidence of *Toxocara* spp. eggs in dog faeces collected from sandpits in Bratislava and in three towns in western Slovakia

Bratislava – district of the city	Sandpits	Animal faeces in the sandpits		*Toxocara* spp. in faeces		*Toxocara* spp. in sand		*Toxocara* spp. total	
	No.	No.	%	No.	%	No.	%	No.	%
Old Town	11	2	18.2	2	100	0	0	2	18.2
D. N. Ves	33	20	60.6	7	35.0	5	15.2	12	36.4
Lamač	19	3	15.8	2	66.7	1	5.3	3	15.8
Total	63	25	39.7	11	44,0	6	9.5	17	**27.0**
Towns in western Slovakia									
Malacky	23	0	0	0	0	0	0	0	0
Pezinok	21	2	9.5	2	100	0	0	2	9.5
Stupava	14	1	7.1	1	100	1	7.1	2	14.2
Total	58	3	5.2	3	100	1	1.7	4	**6.8**

Table 2. *Toxocara* spp. eggs in fenced and unfenced sandpits in Bratislava

Sandbox	Total	*Toxocara* spp.	
		No.	(%)
Fenced	38	12	31.6
Unfenced	25	5	20.0
Examined total	63	17	27.0

Parasitological Laboratory of the State Veterinary and Food Administration in Bratislava on a daily basis.

Enzyme-linked immunosorbent assay for determination of specific antibody production

Sera of pregnant women were examined by ELISA using excretory-secretory antigen for the detection of IgG antibodies against *Toxocara canis*. Serological examinations were evaluated on the level of specific antibodies, corresponding to the relevant values of absorbance at 492 nm: low values 0.600 – 0.900 nm; medium values 0.901 – 1.200 nm and high values 1.201 nm and more (Havasiová *et al*., 1993).

Statistical analysis

The results were analysed with the χ^2 test (Kmety *et al*., 1983).

Results

In Bratislava, out of 63 examined sandpits, *Toxocara* eggs were detected in 17 (27 %) of them. The eggs were present dispersed in the sand and randomly collected faeces; the occurrence of eggs of *Toxocara* spp. in particular districts correlates with the presence of faeces in the sandboxes. The highest contamination with *Toxocara* eggs was found in Devínska Nová Ves (36.4 %); the eggs were detected in 5 sandpits (15.2 %) and7 samples of the faeces (35.0 %) present in the sandpits. The significantly highest contamination of sandpits with dog faeces (60.6 %) was recorded in comparison to their occurrence in the Lamač district (15.8 %; $\chi^2 = 9.82$, $P \leq 0.01$), and equally in the Old Town district (18.2 %, $\chi^2 = 5.94$, $P \leq 0.05$). In three towns in Western Slovakia, out of 58 sandpits, eggs of *Toxocara* spp. were found in the towns of Pezinok and Stupava only; where the eggs of ascarids were present only in three sandpits (6.8 %): in two samples of faeces found in a sandpit in Pezinok, in Stupava the eggs were present in a single sandbox in faeces and the sand. In Malacky, no faeces and no eggs were found in any of the investigated sandpit (Tab. 1).

In the districts of Bratislava, out of 63 sandpits, 38 (60.3 %) were fenced, and 25 (39.7 %) unfenced. *Toxocara* eggs were found in 12 fenced sandpits (31.6 %). Surprisingly, ascarid eggs were found only in 5 unfenced sandpits (20.0 %), but the difference was statistically insignificant ($\chi^2 = 1.03$, $P \leq 0.05$) (Tab. 2).

During 2006 – 2010, faecal samples of 1436 dogs and 263 cats were investigated. *T. canis* eggs were found in the faeces of 16.5 % of dogs and *T. cati* in 18.6 % of examined

Table 3. Coprological findings of parasites in dog faeces

Town	Bratislava		Malacky, Pezinok, Stupava		Total	
	No.	(%)	No.	(%)	No.	(%)
Examined total	621	43.2	815	56.8	1436	100.0
Positive	291	46.8	474	50.3	613	42.7
Toxocara canis	80	12.9	157	19.2	237	16.5
Strongyloides spp.	8	1.3	18	2.2	26	1.8
Trichuris vulpis	9	1.5	14	1.7	23	1.5
Ancylostoma caninum	18	2.9	17	2.1	35	2.4
Uncinaria spp.	1	0.2	2	0.2	3	0.1
Taenia sp.	3	0.5	-	-	3	0.1
Diphylidium caninum	1	0.2	-	-	1	0.07
Mesocestoides	-	-	1	0.1	1	0,07
Giardia spp.	99	15.9	158	19.4	257	17.8
Isospora spp.	60	9.7	101	12.4	161	11.2
Demodex spp.	10	4.9	6	0.7	16	1.11
Cheyletiella yasguri	2	0.3	1	0.1	3	0.01

Table 4. Coprological findings of parasites in cats faeces

Town	Bratislava		Malacky, Pezinok, Stupava		Total	
	No.	(%)	No.	(%)	No.	(%)
Total of examined	114	54.5	149	45.5	263	100
Positive	46	40.4	83	55.7	129	49.1
Toxocara cati	20	17.5	29	19.4	49	18.6
Strongyloides spp.	2	1.8	7	4.7	9	3.4
Toxascaris leonina	-	-	1	0.7	1	0.4
Taenia spp.	-	-	1	0.7	1	0.4
Toxoplasma gondii	1	0.9	2	1.3	3	1.1
Isospora spp.	14	12.2	25	16.8	39	14.8
Giardia spp.	9	7.9	17	11.4	37	14.1

cats (Tab. 3, 4). The difference in the prevalence of ascarid worms in dogs and cats was not statistically significant (χ^2 = 0.67, P ≤ 0.05). Substantially higher prevalence of *T. canis* was detected in dogs bred in towns outside Bratislava than was that in dogs in Bratislava (χ^2 = 10.88, P≤ 0.001). The difference in prevalence of *T. cati* in cats bred in Bratislava and outside Bratislava (P ≤ 0.05) was not confirmed.

Another 12 parasite species were detected in dogs, eight helminth species and four parasitic protozoans. The most frequent parasite was *Giardia* spp., which occurred at the same rate (17.8 %) as *Toxocara canis* (16.5 %) and protozoan parasite *Isospora* spp., present in 11.2 % of the dogs. Significant differences between other parasites in infected dogs in and outside Bratislava were not observed. Other parasites occurred sporadically (Table 3).

Besides *Toxocara* eggs, the faeces of examined cats contained another three species of parasitic worms and cysts of three protozoan species. The most abundant species were coccidian *Isospora felis* (14.8 %) and *Giardia intestinalis* (14.1 %). As for the helminths, *Strongyloides* spp. was found in nine cats (3.4 %). *Toxascaris leonina* and *Taenia* spp. occurred occasionally in cats originating from towns outside Bratislava (Table 4).

Out of 382 pregnant women, anti-*Toxocara* antibodies were detected in 32 females (8.4 %). In Bratislava, 15 s eropositive females were detected (6.6 %); in the towns outside Bratislava, the number reached 17 females (11.0 %) (Tab. 5). The difference was not statistically significant (χ^2 = 1.6, P≤0.05). In the majority of the cases, low titres of antibodies (53.1 %) were detected. Threshold and middle titres were represented with equal frequency (27.6 %, respectively. 21.9 %) (Table 6).

Table 5. Anti-*Toxocara* IgG antibodies detected in sera of pregnant women

Examined total	Positive		Bratislava			Malacky, Pezinok, Stupava		
	No.	(%)	Total	Positive No.	(%)	Total	Positive No.	(%)
382	32	8.4	228	15	6.6	154	17	11.0

Discussion

In humans, the larvae of cat and dog roundworms cause severe disease occurring worldwide. An increasing number

Table 6. The frequency of IgG antibody titers against *Toxocara* spp. in pregnant women (n = 32)

Titre	Bratislava		Malacky, Pezinok, Stupava		Total	
	No.	%	No.	%	No.	%
Cut off titre (1: 100)	4	26.7	4	23.5	8	25.0
Low titre (1: 200)	10	66.7	7	41.2	17	53.1
Middle titre (1: 400 – 800)	1	6.7	6	35.3	7	21.9

of cats and dogs in urban and rural agglomerations, the majority of them not being in the care of a veterinarian, as well as, close contact of man to the contaminated environment pose the risk of infection. In Slovakia, larval toxocariasis is the most commonly diagnosed tissue helminthosis. In epidemiology of larval toxocariasis, the contamination of the environment with *Toxocara* eggs is a key factor. There are numerous studies, documenting the different status in the occurrence of roundworms in different cities. In Košice (Slovak Republic), Juriš *et al*. (1991) reported eggs of *Toxocara* spp. in 18.8 % of sandpits, in faeces and the soil of public areas. In Prague (Czech Republic), Dubná *et al*. (2007) found 20.4 % of sandpits contaminated with *Toxocara* eggs. In Krakow (Poland), Mizgajska (2000) detected *Toxocara* eggs in 30 % of the soil samples from public places. High contaminations of sandpits (84 %) were detected by Duwel (1984) in Frankfurt am Main, Germany. In Greece, Himonas *et al*. (1992) found 97.5 % of children playgrounds contaminated with roundworm eggs. Abe and Yakusava (1997) in Osaka (Japan) found eggs of *Toxocara* spp. in 75% of children sandpits.

Our results provided an interesting overall picture. While, in selected urban areas of Bratislava, we recorded roundworm eggs in up to 27 % of children playgrounds, in the smaller towns outside of Bratislava *Toxocara* eggs were found only in 6.8 % of sandpits. In Bratislava, we found an alarmingly high percentage of sandpits contaminated by dog faeces (39.7 %). Faeces occurred both on the surface of the sand and in deeper layers. *Toxocara* spp. Eggs were present in 44 % of faeces samples, which is more than twice as many samples as found in other parts of Bratislava by Totková *et al*. (2006) (18.7 %). Similarly, Komžíková (1997) in Prievidza found *Toxocara* spp. eggs in 19 % of faecal samples collected from public spaces.

The highest contamination of sandpits with roundworm eggs and faeces was observed in the outskirts of Bratislava – in Devínska Nová Ves. In the cities outside Bratislava, dog faeces contaminated with eggs of *Toxocara* spp. were found only in two sandboxes in Pezinok and one in Stupava. In Malacky, faeces or eggs were not found in any of the sandpits. Supposedly, it might be due to careful treatment of sand only in the cities and responsible approach of pet owners that regularly collect their pet faeces within those areas.

Although the selected sandpits and public areas contained only dog faeces, the high prevalence of *Toxocara* spp. in the district of D. N. Ves may be due to stray cats. It will be necessary to confirm such assumption in order to take the preventive measures. Uga (1996) found that cats tend to disperse their faeces around the sandpits, which contributes most to the subsequent contamination.

This phenomenon could explain the absence of cat faeces in our samples, which were not found in any of the sandpits. Moreover, such assumption could be confirmed by the presence of ascarids eggs in fenced sandpits that do not prevent a cat from entering the playground. In fenced sandboxes, we detected eggs of *Toxocara* spp. relatively more frequently (31.6 %) than in unfenced (20 %). We confirmed the findings of Abe and Yasukawa (1997), and Uga and Kataoka (1995) that a fence will not prevent the sandpits from further contamination. *T. canis* and *T. cati* eggs are morphologically hardly distinguishable (Dubna *et al*., 2007), so we were unable to determine the proportion of cats and dogs on the contamination of sandpits with ascarids. For the species determination of the eggs, methods of molecular differentiation of PCR (polymerase chain reaction) and LAMP (loop-mediated isothermal amplification) appeared appropriate (Borecká & Gawor, 2008, Mačuhová *et al*., 2010).

The low contamination of sandpits with roundworm eggs in the towns outside Bratislava is in contrast to the relatively high prevalence of toxocariasis in dogs and cats (dogs – 19.2 %, cats – 19.4 %) bred in these towns. Infectivity of those cats is comparable to the infectivity of cats kept in Bratislava (17.5 %), while, in dogs, it was significantly higher in the dogs from the cities outside Bratislava that in Bratislava dogs ($P < 0.001$). We assume that it is associated with free movement, the possibility to catch and eat small rodents, which may influence the infection of dogs and cats in rural areas.

In Hanover, on the contrary, Mundhenke & Daugschies (1999), found a higher prevalence of *Toxocara cati* in cats living in rural areas (7.9 %) than was that in urban areas (4.7 %). The high detected amount of dogs (16.5 %) with canine ascarids corresponds to literature data published in Slovakia: Antalíková (1995) found *T. canis* in 10.5 % dogs, Antolová *et al*. (2004) reported *T. canis* infection in 16.6 % of dogs. In 32 villages of South Moravia, Borkovcová (2003) found roundworms in 9.5 % of adult dogs and 22.2 % of puppies. In Poland, Luty (2001) indicated up to 32.0 % occurrence of *T.canis* in dogs. Up to 97.3 % prevalence of *Toxocara canis* in dogs was recorded in Bulgaria (Šoilev *et al*., 1984). Due to the fact that even as low as 7 percent prevalence of *Toxocara* infection in dogs may pose a health risk to humans (Preiss, 1982), our data can be considered relevant in terms of epidemiology of larval toxocariasis. In cats, we found a higher prevalence of toxocariasis than in dogs (18.6 %, respectively 16.5 %), but those values were not statistically significant. However, it is significantly lower prevalence than indicated by Antalíková (1995), who found roundworms in 44.5 % of cats during 1973 – 1994. Similar values in cats (39.0 %) in Poland were reported by Luty (2001). In contrast, our data correspond to the findings of Borkovcová (2002) in the Czech Republic, who found ascarids in 14.2 % of cats. High prevalence of roundworms in cats (52.8 %) was documented in Iran (Sadjjadi *et al*., 2001).

In addition to eggs of *Toxocara* spp. detected in the fecal samples, several other parasite species were found in dogs. *Giardia* spp. was as frequent (17.8 %) as *T. canis* (16.5 %). The occurrence of *Isospora* spp. was significantly lower in dogs than was that in cats (11.2 %, respectively 14.8 %, $\chi 2 = 9{:}48$ for $P < 0.01$). Besides the eggs *T. cati*, six additional species of parasites were detected in cats from Bratislava and smaller towns close to Bratislava; dominated by parasitic protozoans *Isospora* spp. and *Giardia* spp. Simi-

larly, a high prevalence of both significant zoonotic parasites in dogs and cats was detected in the Netherlands (Overgaauw, 2009) and in dogs in Belgium and Japan (Claerebout *et al*., 2009, Naoyuki *et al*., 2009).

Despite the high prevalence of toxocariasis in dogs and cats, as well as high environmental contamination with eggs of *Toxocara* spp. in Bratislava, the seroprevalence of toxocariasis in healthy pregnant women from Bratislava showed relatively low values (6.6 %). We also found a similar occurrence of antibodies in pregnant women in Malacky Pezinok and Stupava (11.0 %).

Lower seroprevalence of toxocariasis is documented by Pavlínová *et al*. (2011) in pregnant women with a history of habitual abortion (5.5 %). Low prevalence of toxocariasis in pregnant women (3.1 %) (samples of the healthy population) was recorded in the Danish population by Stensvold *et al*. (2009).

The occurrence of toxocariasis varies by region, population group, and depends on hygiene practices, and other factors. (Reiterová *et al*., 2003). In Slovakia, Havasiová *et al.* (1993) detected antibodies in 13.7 % of blood donors. Similarly Jalili *et al*. (1997) proved the seropositivity in 15.2 % of blood donors from West Slovakia. In contrast, Savigny *et al*. (1979) in England documented seropositivity only in 2.6 % of blood donors. In Sweden, Ljungström and van Knapen (1989) found antibodies in 7.0 % of the healthy population; in Turkey, Kaplan *et al*. (2004) also in 7.1 % of healthy persons. The highest seroprevalence – 86 % is recorded in Saint Lucia, in the Caribbean area (Safar *et al*., 1990). Alderete *et al*. (2003) in Brazil found antibodies against *Toxocara* spp. in 38.8 % of healthy children and observed a statistically significant correlation between occurrence of antibodies and low hygiene practices. Fan *et al*. (2004) reported up to 75.6 % of seropositive healthy children living in Taiwan, in an environment contaminated by eggs of *Toxocara* spp. Kaplan *et al*. (2004) detected a substantially higher seroprevalence in groups of mentally disabled persons (18.8 %) than was that in a sample of healthy individuals (7.1 %).

Many authors indicate higher seroprevalence in the rural areas in comparison to towns. Uhlíková and Hübner (1983) in the Czech Republic observed a substantially higher seroprevalence (33.7 %) in rural population than in the towns (13.2 %). Significantly higher seropositivity in the urban population (9,2 %) in comparison to people living in rural areas (15.5 %) is documented by Ondriska (1997).

The results of our study suggest the need for paying permanent attention to larval toxocariasis. A high level of *Toxocara* infection in dogs and cats in above-mentioned cities, and seroprevalence in healthy adult humans refer to inadequate prevention practices.

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