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Epidemiological risks of endoparasitoses spread by municipal waste water

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Summary

The occurrence of developmental stages of endoparasite germs (cysts, oocysts, protozoa, and helminth eggs) as an indirect detection factor of endoparasitoses circulation in the environment, was examined in raw municipal wastewater, sludge and biologically cleaned waste water. Examination of municipal wastewater and sludge from five monitored wastewater treatment plants (WWTPs) in east Slovakia, from various fractions of municipal wastewater, confirmed 35.87 % positivity of samples for the endoparasitic germs. Among of all analysed samples 11.09 % were protozoan oo(cysts) and 20.87 % were helminth eggs. 3.91 % of samples showed positivity to both the helminth eggs and protozoan oo(cysts). In the raw wastewater the protozoa comprised of *Giardia* spp. (1.08 %) and *Entamoeba* spp. (1.08 %). The helminth eggs primarily consisted of *Ascaris* spp. (4.35 %) and strongyle-type eggs (3.26 %). No germs of protozoa or helminths were found in the treated wastewater. However, the highest presence of the germs was found in drained stabilised sludge. The average number of oo(cysts)/kg was 2.86±0.24 and the average number of helminth eggs/kg was 5.77±0.09. In all kinds of sludge, obtained during the process of wastewater treatment, there were protozoan (*Giardia* spp., *Cryptosporidium* spp., *Entamoeba* spp.) and helminths eggs (*Ascaris* spp., *Trichuris* spp., *Taenia* spp., *Hymenolepis* spp., or strongyle-type eggs) presented. In drained (condensed) stabilised sludge the eggs of *Capillaria* spp. and *Toxocara* spp. were also detected. From the epidemiological aspect the sewage sludge, due to high concentration of protozoal oo(cysts) or helminth eggs, represents a significant epidemiological risk for the endoparasitoses dissemination.
Keywords: oo(cysts) of protozoa; helminth eggs; municipal waste water; sewage sludge; epidemiology of endoparasitic germs

Introduction

Prevalence of endoparasitoses, due to an increase in the density of world population, has an increasing tendency not only in developing countries but due to the migration pressure also in developed countries including the EU. This in general represents a significant public-health problem. Intestinal infections induced by protozoa and helminths affect about 3.5 milliard people and they induce diseases in 450 million people worldwide where mostly children are affected (WHO, 2008). The most frequent incidence of parasitic diseases is in the poorest regions with low socio-economical and living conditions, without hygienic and sanitary systems, and possibly without access to the clean drinking water. The environmental conditions are the determining factors in the transmission and dissemination of endoparasitoses in human population. Wastewater tends to be a source of pathogenic microorganisms including the endoparasitic germs. It represents a significant risk of the environmental contamination. Environmental conditions such as contamination of soil and water sources with municipal waste as well as the absence of wastewater treatment and subsequent utilization for fertilization and irrigation purposes contribute to the development and distribution of parasitic infections (Okojokwu *et* *al*., 2014). In Slovakia the incidence of endoparasitoses is especially linked with communities with low hygienic standards and without proper infrastructure. From the epidemiological point of view there are still some relatively risky communities. Those are villages and small urban settlements lacking sewerage and wastewater treatment systems (Rimárová, 2010; Juriš & Papajová, 2012). The monitoring goal was to determine the current situation regarding the incidence of germs of endoparasites in municipal wastewater and sludge from WWTPs. The analysis was associated with subsequent assessment of the epidemiological risk for the public health.

Material and Methods

The samples of municipal wastewater and sludge were collected from five wastewater treatment plants (WWTPs) using the technology of mechanical-chemical-biological water purification. River Torysa was the water source for the examined WWTPs located in Torysa, Lipany, Sabinov and Prešov. For comparison, we also monitored the WWTP Sečovce in another region whose water source is the rivulet Trnávka. WWTPs differed in the number of corresponding inhabitants: 86,998 (WWTP Prešov), 32,400 (WWTP Prešov), 11,405 (WWTP Sečovce), 10,000 (WWTP Lipany), 8,500 (WWTP Torysa). The incidence of germs of endoparasites (the presence of oo(cysts) of protozoa, and helminth eggs) was examined in raw wastewater, biologically treated wastewater, raw sludge, activated sludge and drained stabilised sludge. Totally 184 samples of wastewater and 276 samples of sludge were examined. The sample tested consisted of 250 ml of wastewater, raw and activated sludge or 250 g of drained stabilised sludge. For the detection the oo(cysts) aqueous $ZnSO_4$ solution (Bartlett *et al*. 1978) and eggs method according Cherepanov (1982) were used. The method consisted of sedimentation, centrifugation with sacharose where the specific weight for saturated solution was 1.30 for both, the liquid and solid fractions. Subsequently, the flotation samples were examined under the microscope. The biochemical analysis of raw and treated municipal wastewater was carried out in accordance to the valid Slovak technical standards and parameters of wastewater contamination listed under the Government regulation No. 269/2010 and in accordance with the Regulation of European Parliament and European Council (EC) No. 1137/2008 on the municipal wastewater treatment. For the statistical processing of the results the Chi square test- χ^2 test (significance level α=0.05) the SPSS version 20 of statistical program was employed.

Table 1. Positive samples of municipal wastewater and sludge to the germs of endoparasites (%)

Germs of endoparasites	N/460	P (%)
Protozoa / helminth eggs	18	3.91
Protozoan oo(cysts)	51	11.09
Helminth eggs	96	20.87
Σ posit. samples	**165**	**35.87**
Negative samples	295	64.13

N- number of examined samples, P- positivity

Results

Analysis of 460 wastewater and sludge samples from WWTP revealed 35.87 % positivity to the germs of endoparasites. The oo(cysts) of protozoa were recorded in 11.09 % of samples, and helminth eggs were present in 20.87 %. In 3.91 % of samples there was positivity to both the helminth eggs and protozoan oo(cysts) (Table 1).

Out of the total 92 raw wastewater samples examined 2 were found positive (2.17 %) for the presence of protozoan oo(cysts); genus representation *Giardia* spp. (1.08 %) and *Entamoeba* spp.

Table 2. Species representation of the germs of endoparasites in municipal wastewater and sludge from the WWTPs

Generic or species representation of endoparasites	RW inflow (N=92)	TW outflow (N=92)	Raw sludge (N=88)	Activated sludge (N=88)	Drained stabilised sludge (N=100)
	n posit./P %				
Giardia spp.	1/1.08	0	11/12.50	7/7.95	4/4.00
Cryprosporidium spp.	0	0	1/1.14	2/2.27	3/3.00
Entamoeba spp.	1/1.08	0	2/2.27	1/1.14	1/1.00
Isospora spp.	0	0	0	0	7/7.00
Ascaris spp.	4/4.35	0	17/19.32	11/12.50	48/48.00
Strongyle-type eggs	3/3.26	0	11/12.50	6/6.82	27/27.00
Trichuris spp.	0	0	2/2.27	2/2.27	9/9.00
Capillaria spp.	0	0	0	0	4/4.00
Toxocara spp.	0	0	0	0	2/2.00
Taenia spp.	0	0	9/10.23	2/2.27	9/9.00
Hymenolepis spp.	0	0	2/2.27	1/1.14	2/2.00

RW- raw wastewater, TW- treated wastewater, N- number of examined samples, n posit. - number of positive, P- positivity

Table 3. Germs of endoparasites in the raw wastewater from the WWTPs

WWTP	Number of samples (N)	Oo(cysts) of protozoa				Helminth eggs			
		n (%)	Number of oo(cysts)/l	$\bar{x}$ ± SD oo(cysts)/l	χ^2 (p) RW/TW	n (%)	Number of eggs/l	$\bar{x}$ ± SD of eggs /l	χ^2 (p) RW/TW
WWTP Torysa	12	0	0	0	-	0	0	0	-
WWTP Lipany	20	0	0	0	-	3 (15.00)	8	2.13 ± 0.71	**0.0488***
WWTP Sabinov	24	2 (8.33)	0.83	1.66 ± 0.83	0.1861	3 (12.50)	4	0.88 ± 0.29	0.1022
WWTP Prešov	20	0	0	0	-	1 (5.00)	5	0.20 ± 0.20	0.3619
WWTP Sečovce	16		0	0	-	0	0	0	-
∑	**92**	**2 (2.17)**	**0.16**	**0.33 ±0.08**		**7 (7.61)**	**3.4**	**0.62 ± 0.08**	

WWTP- wastewater treatment plant, N- number of examined samples, n- number of positive samples, $\bar{X}$ ± SD- the average number and standard deviation, χ^2 test, p value expressing statistically significant difference in the presence of germs (endoparasites) between raw wastewater (RW) and treated wastewater (TW). Significance level: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

(1.08 %). The mean number of oo(cysts) per liter ($\bar{x}$ ± SD/l) was 0.33± 0.08. Seven raw wastewater samples were positive for the presence of helminth eggs (7.61 %) where the species *Ascaris* spp. (4.35 %) and strongyle-type eggs (3.26 %) were detected. The mean number of helminth eggs per a liter of raw wastewater ($\bar{x}$ ± SD/l) was 0.62±0.08. All 92 samples collected from treated wastewater were tested negative for the presence of endoparasitic germs. In WWTP Lipany the statistically significant difference (χ^2, p≤ 0.05) in the incidence of the helminth eggs was found (Table 2 and 3).

Out of 88 raw sludge samples the presence of protozoal oo(cysts) was confirmed in 12 of them (13.63 %). The species of *Giardia* spp. (12.50 %), *Entamoeba* spp. (2.27 %), and *Cryptosporidium* spp. (1.14 %) were confirmed. The mean number of oo(cysts) per liter ($\bar{x}$ ± SD/l) was 1.64±0.14. The eggs of *Ascaris* spp. (19.32 %), strongyle-type eggs (12.50 %), *Trichuris* spp. (2.27 %), *Taenia* spp. (10.23 %) and *Hymenolepis* spp. (2.27 %) were present in 32 samples of raw sludge (36.36 %). The mean number of helminth eggs per liter ($\bar{x}$ ± SD/l) in raw sludge was 3.05±0.09. The statistically significant difference (p≤ 0.05) in the presence of the endoparasitic germs was found between raw wastewater and raw sludge. The occurrence of protozoa was significantly higher in raw sludge of WWTP Lipany and WWTP Prešov. The presence of helminths eggs was significantly higher in raw sludge WWTP Prešov and WWTP Sečovce (Tab. 2 and Tab. 4).

Out of the total 88 samples from activated sludge eight samples were positive for the presence of protozoan oo(cysts) (9.09 %). Generically *Giardia* spp. (7.95 %), *Cryptosporidium* spp. (2.27 %), and *Entamoeba* spp. (1.14 %) were identified. The mean number of oo(cysts) per a liter ($\bar{x}$ ± SD/l) was 1.88±0.24. The number of positive samples for the presence of protozoa was the lowest of all kinds of sludge. Helminth eggs were present in 16 samples (18.18 %) and the mean number of helminth eggs per liter ($\bar{x}$ ± SD/l) in activated sludge was 2.008± 0.13, what also represents the lowest value. According to the generic or species representation following egg types were detected: *Ascaris* spp. (12.50 %), strongyle-type eggs (6.82 %), *Trichuris* spp. (2.27 %), *Taenia* spp. (2.27 %), *Hymenolepis* spp. (1.14 %). Based upon the χ^2 test analysis the statistically significant difference in the presence of protozoa in WWTP Sečovce between raw wastewater and activated sludge was confirmed. There was significantly higher number of positive samples in activated sludge. The presence of helminth eggs was also significantly higher in activated sludge from WWTP Torysa and Sečovce (Table 2 and Table 5).

Out of the total 100 examined samples of drained stabilised sludge 12 samples were positive for the presence of protozoa

Table 4. Germs of endoparasites in raw sludge from the WWTPs

WWTP	Number of samples (N)	Oo(cysts) of protozoa				Helminth eggs			
		N (%)	Number of oo(cysts)/l	$\bar{x}$ ± SD oo(cysts)/l	χ^2 (p) RW/TW	n (%)	Number of eggs/l	$\bar{x}$ ± SD eggs /l	χ^2 (p) RW/TW
WWTP Torysa	12	0	0	0	-	1 (8,33)	0.25	0.33 ± 0.33	0.3070
WWTP Lipany	16	3 (18.75)	1.75	2.33 ± 0.77	**0.0429***	6 (37.50)	7.75	5.16 ± 0.86	0.1213
WWTP Sabinov	20	2 (10.00)	1.8	3.60 ± 1.80	0.8497	7 (35.00)	8.0	3.66 ± 0.52	0.0767
WWTP Prešov	24	7 (29.16)	4.0	2.28 ± 0.33	**0.0085****	8 (33.33)	6.0	2.00 ± 0.25	**0.0204***
WWTP Sečovce	16	0	0	0	**-**	10(62.50)	10.25	4.10 ± 0.41	**0.0045****
∑	**88**	**12 (13,63)**	**1.51**	**1.64 ± 0.14**		**32 (36.36)**	**6.45**	**3.05 ± 0.09**	

Symbols used – see legend in Table 3

Table 5. Germs of endoparasites in activated sludge from the WWTPs

WWTP	Number of samples (N)	Oo(cysts) of protozoa				Helminth eggs			
		n (%)	Number of oo(cysts)/l	$\bar{x}$ ± SD oo(cysts)/l	X^2 (p) RW/TW	n (%)	Number of eggs/l	$\bar{x}$ ± SD of eggs /l	X^2 (p) RW/TW
WWTP Torysa	16	1 (6.25)	0.75	0.75 ± 0.75	0.3764	6 (37.50)	2.5	1.66 ± 0.28	**0.0168****
WWTP Lipany	16	2 (12.50)	1.75	3.50 ± 1.75	0.1014	2 (12.50)	1.0	2.00 ± 1.00	0.8274
WWTP Sabinov	24	1 (4.16)	0.67	0.66 ± 0.66	0.5509	2 (8.33)	0.67	1.33 ± 0.67	0.6366
WWTP Prešov	16	0	0	0	-	1 (6.25)	0.5	0.25 ± 0.25	0.8652
WWTP Sečovce	16	4(25.00)	3.75	4.50 ± 1.13	**0.0001 *****	5(31.25)	6.0	4.80 ± 0.96	**0.0360***
∑	**88**	**8 (9.09)**	**1.38**	**1.88 ± 0.24**		**16 (18.18)**	**2.13**	**2.008 ± 0.13**	

Symbols used – see legend in Table 3

(12.00 %). Similarly to in raw or activated sludge there were *Giardia* spp. (4.00 %), *Cryptosporidium* spp. (3.00 %), *Entamoeba* spp. (1.00 %), and *Isospora* spp. (7.00 %) oo(cysts) present.
The mean number of oo(cysts) per a kilogram ($\bar{x}$ ± SD/kg) was 2.86± 0.24. This was the highest quantity when compared with the other kinds of sludge. In comparison to the other types of sludge the presence of helminth eggs in drained stabilised sludge was at the highest level (58.00 %). The mean number of eggs per a kilogram ($\bar{x}$ ± SD/kg) was also the highest (5.77±0.09) as well. The predominant eggs were *Ascaris* spp. (48.00 %) and strongyle-type eggs (27.00 %). Out of the other helminth eggs detected there were *Trichuris* spp. (9.00 %), *Capillaria* spp. (4.00 %), *Toxocara* spp. (2.00 %), from cestodes *Taenia* spp. (9.00 %), and *Hymenolepis* spp. (2.00 %). The quadrate Chí test confirmed the statistical difference between raw wastewater and drained stabilised sludge in all examined WWTPs. The presence of helminths was significantly higher in drained stabilised sludge from WWTP Torysa, WWTP Lipany, WWTP Sabinov, WWTP Prešov and WWTP Sečovce (Table 2 and Table 6).
Biochemical burden was observed in the raw and treated wastewater of monitoring municipal wastewater treatment plants. The list of the selected biochemical indicators is summarized in Tab.7. Biochemical parameters determined in laboratory examination corresponded with standards limit values. The process of purification of waste water was carried out in optimal mode. Tab. 7 shows the average values of biochemical markers during the entire monitored period.

Discussion

The municipal wastewater before treatment contains a large amount of pathogenic microorganisms. Out of them the agents of endoparasitic infections represent a serious risk threat to the public health (Mahvi & Kia, 2006). A lot of studies refer on the presence of endoparasites in raw wastewater. Most common are protozoa such as *Giardia* spp., *Cryptosporidium* spp., *Entamoeba histolytica* (Khouja *et al*., 2010), *Cyclospora cayetanensis,* (Galván *et al*., 2013), *Isospora* spp., *Toxoplasma gondii* (Demar *et al*., 2007) and helminths corresponding to *Ascaris lumbricoides, Trichostrongylus* spp., *Enterobius vermicularis, Ancylostoma duodenale, Necator americanus, Taenia* spp.*, Hymenolepis nana* (Mahvi & Kia, 2006; Ben Ayed *et al*., 2009; Okojokwu *et al*., 2014, Schaechter, 2009).
In wastewater monitored for the presence of the endoparasitic germs we have found hygienically significant contamination. Thus

Table 6. Germs of endoparasites in drained stabilised sludge from the WWTPs

WWTP	Number of samples (N)	Oo(cysts) of protozoa				Helminth eggs			
		n (%)	Number oo(cysts)/kg	$\bar{x}$ ± SD oo(cysts)/kg	X^2 (p) RW/TW	n (%)	Number of eggs /kg	$\bar{x}$ ± SD eggs /kg	X^2 (p) RW/TW
WWTP Torysa	16	4 (16.66)	4.00	4.00 ± 1.00	0.0618	9 (56.25)	8.50	3.77 ± 0.42	**0.0016*****
WWTP Lipany	24	0	0	0	-	13 (54.16)	31.83	9.79 ± 0.75	**0.0071****
WWTP Sabinov	24	3 (12.50)	1.50	2.00 ± 0.66	0.6366	17 (70.83)	24.50	5.17 ± 0.30	**0.0001*****
WWTP Prešov	20	5 (25.00)	10.40	8.32 ± 1.66	**0.0168 ****	13 (65.00)	17.80	5.48 ± 0.42	**0.0001*****
WWTP Sečovce	16	0	0	0	**-**	6(37.50)	6.50	4.66 ± 0.78	**0.0226***
∑	**100**	**12 (12.00)**	**3.18**	**2.86 ± 0.24**		**58 (58.00)**	**17.83**	**5.77 ± 0.09**	

Symbols used – see legend in Table 3

Table 7. Biochemical parameters of municipal waste water contamination before and after the technological treatment in the WWTPs

		WWTP Torysa	WWTP Lipany	WWTP Sabinov	WWTP Prešov	WWTP Sečovce
pH	RW	7.5	7.6	7.8	7.7	7.6
	TW	7.3	7.8	7.6	7.7	7.6
COD (mg/l)	RW	777.2	443.3	883	787.2	289
	TW	20.7	24.3	26.6	30.8	24
BOD (mg/l)	RW	359.8	274.1	567.2	360.3	114
	TW	2.1	2.3	2.2	3.4	7.5
SS (mg/l)	RW	613.8	259.2	343.8	456.8	99.1
	TW	5.2	8.6	5	6.3	5.4
N-NO3^{-} (mg/l)	RW	0.3	0.3	0.4	0.3	1.1
	TW	**5.6***	**6.4***	**6.6***	**3.9***	**20.8***
N-NH4^{+} (mg/l)	RW	34.2	37	44.3	46.5	29.4
	TW	1.9	0.9	0.3	1.9	0.1
P-PO4^{3-} (mg/l)	RW	9	3.4	7.6	6.2	3.5
	TW	0.5	2.7	0.3	0.2	2.6

WWTP- wastewater treatment plant, RW- raw wastewater, TW-treated wastewater, COD- chemical oxygen demand, BOD- biological oxygen demand, SS- suspended solids
Limit values for treated municipal waste water were assessed according to the Government regulation No. 269/2010 and in accordance with the Regulation of European Parliament and Council (EC) No. 1137/2008 on The municipal wastewater treatment.
*values exceeding the limit concentrations in treated waste water

this type of water is unsuitable for direct fertilization or disposal because represents a serious epidemiological risk for the environment. In particular, ground water, sources of drinking water and soil are endangered. For that reason it is necessary to treat contaminated water in the wastewater treatment plants.

A lot of studies have confirmed that treatment does not necessarily remove all the germs of parasites and some of them remain present and are viable. The size of protozoan cysts *Giardia* spp. (8 – 12 μm), *Entamoeba histolytica* (9 – 14.5 μm) and oocysts *Cryptosporidium* spp. (4.5 – 5.5 μm), *Microsporidium* spp. (1.8 – 5.0 μm) permits to persevere throughout the technological processes of wastewater treatment. Similarly to raw untreated wastewater the treated one may pose the same though reduced risk for water and the environment (Slifko *et al*., 2000). For example, Khanum *et al*. (2012) observed the presence of protozoa in municipal wastewater from wastewater treatment plants and confirmed their survival ability during treatment processes. Analysis of treated wastewater validated the presence of protozoa such as *Giardia* spp., *Entamoeba* spp., *Entamoeba coli, Endolimax nana, Idoamoeba butschlii*, and *Balantidium coli.* When samples were collected at the different steps of water treatment the amount of protozoa decreased towards the discharge phase of treatment. However, treatment did not result in complete protozoan removal. Khouja *et al*. (2010) also reported the presence of protozoa *Cryptosporidium* spp., *Giardia* spp., and *Entamoeba* spp. in treated wastewater. In addition, Seviour and Nielsen (2010) pointed out that 20 % of *Giardia* spp. cysts and 33 – 100 % of *Cryptosporidium* spp. oocysts remained in treated wastewater viable or infectious.

Some observations proved the presence of helminth eggs in treated water from the municipal wastewater treatment plants as well. There is constant presence of eggs such as *Ascaris* spp., *Enterobius* spp., *Hymenolepis* spp., and *Strongyloides* spp. (Cutolo *et al*., 2006). However, in our study the presence of the germs of endoparasites in treated water from mechanical-chemical-biological wastewater treatment plants was not confirmed. Therefore it seems that the treatment effectiveness in the municipal wastewater treatment plants examined was from the parasitological aspect fully effective. During the process of wastewater treatment, after the phase of sedimentation, the protozoal (oo)cysts and helminth eggs are accumulated in the sludge (Jiménez *et al*., 2010). Consequently, in the wastewater treatment plants an increased presence of protozoan cysts (*Giardia* spp., *Entamoeba* spp., and oocysts *Cryptosporidium* spp.) in raw sludge is identified. Cheng *et al*. (2009) reported that all the sludge samples analysed from the municipal wastewater treatment plants were positive for the presence of *C. parvum, C. hominis* oocysts and *Giardia intestinalis* cysts. Maximum oocysts concentration was detected in the primary raw sludge and therefore subsequent sanitation is recommended. The oocysts *Cryptosporidium* spp. can remain viable in the environment for more than a year. Therefore protozoan species are most spread in the environment and they can get clearly propagated through their germs.

Guzmán *et al*. (2007) described that the presence of helminth eggs (*Hymenolepis* spp, *Trichuris* spp., *Ascaris* spp.) in raw sludge was at a low concentration while the incidence of *Capillaria* spp. was most frequent. Turovskiy and Mathai (2006) reported a high occurrence of helminth eggs including trematodes, nematodes and cestodes. The helminth concentration also depends on the epidemiological situation for the given territory and hygienic level as well. In our study *Ascaris* spp., strongyle-type eggs, *Trichuris* spp., *Taenia* spp., and *Hymenolepis* spp. were regularly found in a raw sludge.

Study of Mara and Horan (2003) identified the presence of protozoa (*Giardia* spp., *Entamoeba* spp., and *Cryptosporidium* spp., spores of microsporidia) and eggs of *Taenia saginata, Ascaris* spp., *Trichuris* spp., *Hymenolepis* spp. in the activated sludge. The concentration of endoparasites in activated sludge is usually low (less than 5 cysts/eggs per a litre). The samples from activated sludge exhibited the lowest concentrations of endoparasitic germs in all kinds of the sludge examined.

Konate *et al*. (2010) published the study, which was focused on the survival of protozoa and helminths in sewage sludge after the process of its stabilisation. Followed by the anaerobic stabilisation the presence of protozoa *Entamoeba coli*, *Entamoeba histolytica*, *Giardia* spp., and helminths *Ascaris lumbricoides*, *Ancylostoma* spp. was confirmed. In the analysed stabilised sludge a wide scale of germs of endoparasites (protozoa, nematodes, cestodes) were also identified. Generally the process of wastewater stabilisation eliminates about 70 – 99 % of the protozoan oo(cysts) and helminth eggs what is represented by the highest amount found in stabilised sludge (Jimenez, 2007). Our results have confirmed this fact. The highest contamination with the endoparasitic germs was recorded in stabilised sludge in 58 % of positive samples.

Sedimentation is an effective technological phase that eliminates especially heavier helminth eggs and to a lesser degree protozoan cysts. Turovskiy and Mathai (2006) focused on the quantification of helminth eggs in municipal wastewater and sludge. Their results pointed out that the number of helminth eggs per kilogram reached in raw and activated sludge several hundreds and in drained stabilised sludge can be extended to several thousands.

The World Health Organisation recommends the concentration ≤ 1 helminth egg/liter in wastewater used for irrigation and less than 1 helminth egg/g of sludge. The most important helminthic species are *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Necator americanus*. The amount of intestinal helminth eggs in municipal wastewater in developing countries can be within the concentration range of 10 to more than 100,000 eggs per a liter of wastewater. In Europe it is 0 – 1 egg per a liter of wastewater (WHO, 2006). The sludge samples analysed showed higher values of the mean number of oo(cysts) and eggs. This is associated with high risk of the environmental contamination and greater threat to the public health. Therefore hygienisation/disinfection of sludge as well as proper handling, disposal of sludge, or further safe use is suggested.

A significant fact is that the germs of parasites (eggs, cysts, oocysts, sporocysts, larvae) are characterised by high tenacity in the environment due to the notably stemming from the construction of germ walls and their biological and physiological properties. Among of protozoa the oocysts of the genera *Cryptosporidium, Toxoplasma* and the helminths thick-walled eggs of *Ascaris, Toxocara, Fasciola, Taenia, Echinococcus* spp. are the most resistant. Under favorable conditions of the external environment germs are able to survive from several months up to the years (Juriš *et al*., 1991, Dubinský *et al*., 2000).

Conclusion

We found that among of the many risks that can endanger public health is potential spreading of wide spectrum of endoparasitoses (*Giardia* spp., *Cryptosporidium* spp., *Entamoeba* spp.*, Isospora* spp.*, Ascaris* spp.*,* strongyle-type eggs, *Trichuris* spp.*, Capillaria* spp.*, Toxocara* spp., *Taenia* spp., and *Hymenolepis* spp.) throughout the processing and handling of municipal wastewater. In comparison with inflowing wastewater to the WWTPs the presence of the developmental stages of protozoa and helminths was significantly higher in both the raw and activated sludge. The presence of helminth eggs (*Ascaris* spp., strongyle-type eggs, *Trichuris* spp.*, Capillaria* spp.*, Toxocara* spp., *Taenia* spp.*,* and *Hymenolepis* spp.) was highly significant in drained stabilised sludge. Therefore the degree of contamination in drained stabilised sludge with the developmental stages of endoparasites (oocysts, helminth eggs) is epidemiologically extremely important. Municipal wastewater treatment plants within the technological process of treatment do not devitalize the developmental stages of endoparasites but concentrate them into various sludge fractions. In conclusion, the sludge fractions represent potential reservoirs for the endoparasitic diseases and epidemiologically should be considered very hazardous. Incorrect manipulation or ineffective sludge sanitation can cause epidemiologically significant contamination of the environment.

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