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Helminth eggs in the sludge from three sewage treatment plants in Czechoslovakia

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Abstract. Developmental stages of 8 species of parasitic helminths were found during examination of sludge from 3 sewage treatment plants in Central Bohemia, Czechoslovakia. The eggs were comprised from *Ascaris* sp., *Toxocara* sp., *Toxascaris leonina, Parascaris equorum, Enterobius vermicularis, Trichuris* sp., *Capillaria* sp. and *Hymenolepis* spp. The quantity of these organisms in 100 g of dried sludge ranged between 24–105 eggs of *Ascaris* sp., 12–47 eggs of *Toxocara* sp., 0–8 eggs of *Toxascaris leonina*, 0–1 egg of *Parascaris equorum*, 2–33 eggs of *Trichuris* sp., 0–12 eggs of *Capillaria* sp., 0–46 eggs of *Enterobius vermicularis* and 0–49 eggs of *Hymenolepis* spp. Some eggs of *Hymenolepis* spp. and *Enterobius vermicularis* were structurally damaged, while some eggs of *Ascaris* sp. and *Enterobius vermicularis* contained developed larvae.

Sewage sludge is one of the products in municipal sewage treatment plants. It has been often used as agricultural fertilizer. However, this practice should be reconsidered due to hygienic problems caused by frequent contamination of the sludge by heavy metals and biological pathogens. Besides viruses and bacteria (Burge et al. 1978), these pathogens are also represented by protozoan cysts and helminth eggs.

The eggs of the following helminths were found by various authors in the sewage and sewage sludge: *Ascaris* sp., *Toxocara* sp., *Taenia saginata* Goeze, 1782, *Taenia solium* Linnaeus, 1758, *Trichuris* sp., *Enterobius vermicularis* (Linnaeus, 1758), *Hymenolepis* sp., *Diphyllobothrium latum* Linnaeus, 1758, *Schistosoma* sp., *Ancylostoma duodenale* (Dubini, 1843) and *Fasciola* sp.

The organisms occurring in sludge differ in their ability to survive adverse conditions during sewage treatment (Schätzle 1969, Forstner 1970, Günthör 1971, Schaffert and Strauch 1976, Hays 1977). Among protozoan parasites probably the most resistant are coccidian oocysts (Geller 1982) and among helminth parasites the eggs of *Ascaris* sp. (Wiley and Wasterberg 1969, Günthör 1971, Fitzgerald and Ashley 1977, Arther et al. 1981). The treatment processes are effective mainly in destroying viruses and bacteria, however, protozoa and helminths in resistant stages often go undestroyed (Hays 1977). Only some authors documented total destruction of parasitic organisms during sewage treatment (achieved e.g. by thermophilic anaerobic digestion (Geller 1982, Pike et al. 1983, Birbaum and Eckert 1985)). Due to the content of potentially viable parasitic stages in sludge there is still one unresolved question: is the sewage sludge really dangerous when disposed freely or can its usage in agriculture be considered as an "acceptable risk"?

This paper reports the qualitative and quantitative findings of eggs of parasitic helminths in five types of sewage sludges examined in Bohemia from 1985 to 1987 and the evaluation of the morphological status of helminth ova that was found.

MATERIALS AND METHODS

The sewage sludge samples were obtained from 3 municipal sewage treatment plants in towns of Kutná Hora, Příbram and Prague (Central Bohemia, Czechoslovakia). Five types of sludges were examined. In Příbram, it was the liquid sludge after anaerobic digestion at 25–26 °C (sludge A; 6 samples) and the sludge after aerobic and concentration steps of treatment (sludge B; 7 samples). In Kutná Hora it was the liquid sludge after activation in ditches (sludge C; 4 samples) and the liquid or concentrated sludge after anaerobic digestion at 33–35 °C (sludge D; 6 samples). In Prague it was the liquid sludge after anaerobic digestion at 38–42 °C (sludge E; 12 samples).

The samples (400 ml each) were taken from the sewage sludge piping system. When the obtained sample contained only a small amount of water, 30 g of sewage sludge material was further processed. From samples which were in liquid form, 150 g was examined. The flotation method of Vasilkovová (1953) and Romanenko (1968) as modified by Sedláček and Stoklasová (1977) was used for the separation of the parasitic elements from other materials in the sample. The final flotation used in this procedure was repeated twice with each sample.

The routine examination of sludge was made using light microscope at low power (120 ×). The morphological and developmental status of eggs that were found in the samples was evaluated at magnification of 450 ×.

To compare the samples from different types of sludges, we calculated the mean number of parasitic organisms per 100 g of dried sludge from all samples obtained during the whole period of the study. The individual samples being non-representative.

RESULTS

We found eggs of 8 species of parasitic helminths: *Ascaris* sp., *Toxocara* sp., *Toxascaris leonina* (Linstow, 1902), *Parascaris equorum* (Goeze, 1782), *Trichuris* sp., *Capillaria* sp., *Hymenolepis* spp. and *Enterobius vermicularis.* The mean number of these organisms in sludge from different treatment plants is shown in Table 1.

The eggs of *Ascaris* sp., in most cases, had their outer albuminous coat preserved, and mostly were unembryonated. In the sludge E from Prague, 15 % of eggs contained larvae and 10 % of eggs were infertile. In sludge C, there was 20 % of infertile eggs. All *Ascaris* sp. eggs looked structurally intact.

The eggs of *Toxocara* sp., *T. leonina, P. equorum, Trichuris* sp. and *Capillaria* sp. were unsegmented and seemed structurally intact.

The eggs of *Hymenolepis* spp. were represented by two groups according to their size: 43–51 μm × 32–41 μm and 59–68 μm × 57–62 μm, respectively. In the first group they were either with or without filaments of the embryophore and in the second group they were without filaments. The eggs with the filaments represented 20 % of *Hymenolepis* spp. eggs found in sludge E, 57 % of eggs in sludge A, 38 % of eggs in sludge B and 100 % of eggs in sludge C. The larger eggs were found only in sludge E. The *Hymenolepis* spp. eggs were damaged in samples after anaerobic digestion and aerobic treatment in Příbram (sludge A and B). The ratio of these damaged eggs was 57 % and 25 %, respectively.

Table 1. The mean number of helminth eggs per 100 g of dried sludge from all samples during the whole period of study

Parasite	Asc.	Tox.	T. l.	P. e.	Tri.	Cap.	Hym.	E. v.
Sludge A	105	43	0	0	6	3	49	0
Sludge B	58	47	8	0	14	0	30	32
Sludge C	40	12	0	0	7	0	11	11
Sludge D	24	20	2	0	33	12	0	46
Sludge E	34	14	0	1	2	9	3	0

Eggs of parasitic helminths: Asc. – *Ascaris* sp., Tox. – *Toxocara* sp., T. l. – *Toxascaris leonina*, P. e. – *Parascaris equorum*, Tri. – *Trichuris* sp., Cap. – *Capillaria* sp., Hym. – *Hymenolepis* spp., E. v. – *Enterobius vermicularis.*

As far as the eggs of *E. vermicularis* are concerned, we found larvae in 50 % of the eggs in sludge C, in 20 % of the eggs in sludge D and in 22 % of the eggs in sludge B. In sludge C 50 % of the eggs, in sludge D 61 % of the eggs and in sludge B 38 % of the eggs looked structurally damaged.

DISCUSSION

We never found eggs of *Taenia* spp. Considering the fact that the examined sludges are from urban agglomerations, one would gather that the eggs of human parasite *Taenia saginata* would more than likely occur in our samples. The fact that no eggs were recovered is apparently due to the low prevalence of *T. saginata* in Bohemia in the recent years.

Vošta (1958) reported that the eggs of *E. vermicularis* and *Hymenolepis nana* Siebold, 1852 cannot survive in waste water and sewage sludge and are quickly destroyed. However, we disagree with this view because we found both damaged and intact eggs of *E. vermicularis* and *Hymenolepis* spp. As far as the undestroyed eggs of these species are concerned, we think that in this case the digestion did not have a sufficient effect on the eggs due to low temperature and/or unfunctional digestion step.

If we hypothesize that the development of helminth eggs is inhibited in the sewage sludge (Schätzle 1969, Forstner 1970, Günthör 1971, Ware 1980) then we can suppose that the development of larvae within the eggs of *E. vermicularis* and *Ascaris* sp. in our samples occurred before reaching the sewage treatment plant and/or in the aerobic activation ditches. This could be true especially in the case of *E. vermicularis* eggs which have short developmental time.

According to our findings of unembryonated thick-shell eggs (with one exception in the sludge E) we believe that in sewage sludge an obvious inhibition of the development or possibly some morphologically undetermined damage took place in these stages.

Due to the low number of eggs per volume in fresh sludge samples and thus difficult manipulation of them in our laboratory conditions, we did not incubate the above mentioned nematode eggs in order to test their viability.

It was difficult to distinguish individual species of some parasitic helminths whose ova were found. Very similar and structurally undistinguishable are eggs within the following genera: *Ascaris, Toxocara, Trichuris, Capillaria* and *Hymenolepis*. We believe, however, that eggs of *Hymenolepis* spp. were mainly those of cestodes found in rats living in sewage drains (specifically of *Hymenolepis diminuta* Rudolphi, 1819 (bigger eggs) and *H. fraterna* Stiles, 1906 (smaller eggs)).

We did not consider in our study thin-shell eggs and larvae of nematodes (probably of soil or plant nematodes) and of mite eggs which all occurred in our samples quite frequently. Also, we did not evaluate our findings of coccidian oocysts found by our microscopic examinations.

We found eight species of parasitic helminths in the examined sludges, some of them probably being from humans. This indicates the potential health risk associated with the agriculture application of sludges. However, it is quite difficult to make qualified evaluation of the actual health importance of those findings. For such evaluation it would be necessary to know the viability of the parasites and the influence of further sludge disposal on the ova. Without this data we can use our results, if they are compared during long-term period, only as indicator for changes in the prevalence of some parasitic diseases and/or as an additional, low weight criterion for regulation of agriculture sludge application.

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