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Project Summary

Investigation of Parasites in Sludges and Disinfection Techniques

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The objectives of this research grant were to: 1) assess the presence and densities of resistant stages of parasites in municipal wastewater sludges (sewage) in northern United States; 2) compare the results of this study with the results of our previous study of sludges in southern United States; 3) evaluate several decontamination techniques for their effectiveness in inactivating parasites in waste sludges; and 4) develop a standard method for the parasitologic examination of waste sludges. Sludge samples from all phases of treatment (i.e., primary, etc.) were collected during the fall, winter and summer from 48 municipal wastewater treatment plants located in New York (13 plants), Ohio (12 plants), Minnesota (11 plants), and Washington (12 plants). Resistant stages of twenty types of parasites were found in these samples which may represent 25 to 30 different human and animal parasites.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The problems associated with the occurrence of human and animal parasites in domestic wastewater sludges have received little attention in the United States prior to 1970. Even though studies had examined wastewater effluents and sludges from municipal treatment plants in different parts of the country and had found the eggs of several intestinal helminths, these studies have little practical value other than recording the presence of the parasites found, since the techniques used to recover the parasites and to quantitate them varied from study to study.

In a recent study for the U.S. Environmental Protection Agency (EPA) by Tulane University, "Parasites in Southern Sludges and Disinfection by Standard Sludge Treatment," the presence and densities of parasites in sludge samples from 27 municipal wastewater plants in Florida, Alabama, Mississippi, Louisiana, and Texas were investigated over one year, and several standard processes were evaluated in regard to their effectiveness in inactivating parasites. The results indicated that further testing was required to develop reliable approaches to the inactivation of parasites in municipal sludges, and that additional information on the occurrence of parasites in sludges in other areas of the United States was needed before the potential health risks associated with the land application of wastewater sludges could be judged.

The purpose of the present study was to determine the types and densities of parasites in sewage sludges in northern United States, to evaluate factors which may affect their densities in final sludges, i.e., sludges destined for final disposal, and, through laboratory experiments to determine the effectiveness of several sludge treatment processes in inactivating parasites.

Field studies consisted of collecting sludge samples representing all phases of the treatment process (primary stabilization, post-stabilization, etc.) during the fall, winter and spring seasons, respectively, from 48 municipal wastewater treatment plants located in New York (13), Ohio (12), Minnesota (11) and Washington (12). Plants of four size categories, <1 MGD, 1-10 MGD, 10-50 MGD, and >50 MGD, were included in the study. Each sample was analyzed for parasites and several abiotic parameters, i.e., pH, temperature, total suspended and volatile solids, etc. In laboratory studies, aerobic digestion, lime and caustic stabilization, ammonification, irradiation with cobalt 60 and ultrasonification were investigated regarding their effectiveness in inactivating parasites in municipal sludges.

Table 1. *Parasites Found in Sludge Samples from 48 Municipal Plants in Northern United States*

Parasite Found	*Probable Identity*	*Definitive Host*
Ascaris *eggs*	Ascaris lumbricoides[1]	*Humans*
	Ascaris suum	*Pigs*
Toxocara *eggs*	Toxocara canis[2]	*Dogs*
	Toxocara cati[2]	*Cats*
Trichuris trichiura *eggs*	Trichuris trichiura	*Humans*
	Trichuris suis[3]	*Pigs*
Trichuris vulpis *eggs*	Trichuris vulpis	*Dogs*
Toxascaris-*like eggs*	Toxascaris leonina	*Dogs and cats*
Parascaris equorum *eggs*	Parascaris equorum	*Horses*
Ascaridia-*like eggs*	Ascaridia galli	*Domestic poultry*
	Heterakis gallinae	*Domestic poultry*
Gongylonema-*like eggs*	Gongylonema neoplasticum	*Rat*
	Gongylonema pulchrum	*Cattle, pigs, etc.*
Physaloptera-*like eggs*	Physaloptera *spp.*	*Dogs, cats, etc.*
	Turgida turgida	*Opossums*
Trichosomoides-*like eggs*	Trichosomoides crassicauda	*Rats*
	Anatrichosoma buccalis	*Opossums*
Capillaria *spp. eggs (3 or more types)*	Capillaria hepatica	*Rats*
	Capillaria gastrica	*Rats*
	Capillaria *spp.*	*Birds*
	Capillaria *spp.*	*Wild mammals (opossums, racoons, etc.)*
Hymenolepis diminuta *eggs*	Hymenolepis diminuta	*Rats*
Hymenolepis nana *eggs*	Hymenolepis nana	*Humans and rodents*
Hymenolepis *sp. eggs*	Hymenolepis *spp. (poss. more than one species)*	*Domestic and/or wild birds*
Diphyllobothrium-*like eggs*	Diphyllobothrium latum	*Humans, dogs, bears*
	Diphyllobothrium *spp.*	*Dogs, bears, birds*
Spirometra-*like eggs*	Spirometra mansonoides	*Dogs and cats*
Schistosoma mansoni *egg (1)*	Schistosoma mansoni	*Humans*
Entamoeba *coli-like cysts*	Entamoeba coli[4]	*Humans*
	Entamoeba *spp.*	*Rodents, etc.*
Giardia *cysts*	Giardia lamblia	*Humans*
	Giardia *spp.*	*Dogs, cats, mammals*
Coccidia *oocysts*	Isospora *spp.*	*Dogs, cats*
	Eimeria *spp.*	*Domestic and wild birds, mammals*

[1]*Eggs of* A. lumbricoides *and* A. suum *are indistinguishable.*
[2]Toxocara *eggs were probably mostly* T. canis.
[3]T. suis *eggs were probably only rarely seen.*
[4]*An intestinal amoeba that is a commensal, not a parasite.*

Results

Field Studies

Parasitological Findings

In sludge samples collected from each of the 48 treatment plants in the four northern states, 20 different types of parasite eggs or cysts were detected (Table 1), but many of them could be identified to the genus level only. Approximately 90% of all sludge samples examined contained stages of one or more parasites. The four most commonly found parasites were *Ascaris* spp., *Trichuris trichiura*, *Trichuris vulpis* and *Toxocara* spp. The eggs of one or more of these four parasites were found in about 89% of the samples examined from the four states. The geometric means of the numbers of eggs per kg dry weight of sludge of each of these four parasites found in different types of sludge samples from the four states and the percentages of samples positive for these parasites are presented in Table 2. The percentages of treatment plants in the four states with samples positive for these eggs are shown in Table 3.

Ascaris eggs were found in a higher percentage of samples in Washington than in the other three states and the mean number of eggs was also higher in Washington sludges. *T. trichiura* eggs were found more commonly in New York than in the other three states. *T. vulpis* eggs were found in more sludge samples in Ohio and New York, but the egg densities did not differ significantly in the sludges in the four states. *Toxocara* eggs were the most frequently found parasite with viable eggs being found in one or more samples from each plant studied (Table 3). The densities of *Toxocara* eggs were considerably lower in samples in Minnesota than in samples in the other three states.

Parasite Densities vs. Population Served by Plants

Utilization of multiple regression analysis to examine the relationship of the population served by the wastewater treatment plants to the densities of total and viable *Ascaris* and *T. trichiura* eggs in undigested sludges were not related to the size of the population served by the plant; however, the densities of total and viable *T. vulpis* and *Toxocara* eggs decreased significantly in the undigested sludges from northern (and/or southern) states as the population served by the treatment plants increased. In digested sludges, the densities of viable and total *Ascaris* and *T. trichiura* eggs increased significantly with an increasing population size, but the densities of viable *T. vulpis* eggs and viable total *Toxocara* eggs were not significantly related to the population served.

Parasites in Stabilized Sludges

In general, no differences were noted in the densities of eggs in aerobically and anaerobically digested sludges except for viable *Ascaris* and *Toxocara* eggs. Significantly lower densities of vi-

Table 2. Ascaris *spp.,* Trichuris trichiura, T. vulpis *and* Toxocara *spp. Eggs in Samples of Undigested Sludge from Digesters*

Source or Type of Sludge	Ascaris spp. Total[1]	Ascaris spp. Viable	Trichuris trichiura Total[1]	Trichuris trichiura Viable	T. vulpis Total[1]	T. vulpis Viable	Toxocara spp. Total[1]	Toxocara spp. Viable
Undigested								
N = 162								
% positive	51%	49%	35%	27%	33%	31%	81%	78%
Geometric Mean[2]	940	670	500	650	430	440	880	870
Digester								
N = 137								
% positive	56%	50%	42%	28%	48%	45%	85%	74%
Geometric Mean[2]	1700	1400	480	400	460	440	1200	740
Post-Digestion								
N = 113								
% positive	54%	45%	29%	19%	47%	37%	69%	50%
Geometric Mean[2]	560	310	260	130	240	190	330	190
All Sludges								
N = 142								
% positive	54%	48%	36%	25%	42%	37%	79%	69%
Geometric Mean[2]	1000	710	440	400	400	370	880	670

[1]Viable and nonviable eggs.
[2]Antilog of the mean of the log of positive samples, expressed as number of eggs/kg dry weight of sludge.

Table 3. *Percentage of Plants in Four Northern States with Eggs of* Ascaris, Trichuris trichiura, T. vulpis *and* Toxocara.

Parasite	*Viable and Nonviable Eggs*	*Viable Eggs*
Ascaris	85%	77%
T. trichiura	88%	73%
T. vulpis	85%	81%
Toxocara	100%	100%

able *Ascaris* and *Toxocara* eggs were found in anaerobically digested sludges.

Parasites in Drying Beds

In all cases a significant relationship between the density of viable eggs and the moisture content of the sample was observed. As the moisture content decreased, the densities of viable parasite eggs decreased.

Step-up multiple regression analysis was applied to the density of viable eggs in drying beds vs. the density of viable eggs in a simultaneously collected sample of undigested sludge. The purpose was to test the significance of the effects of the percent moisture content of the drying bed, the age of the sludge in the bed, the season of the sampling, and the type of digestion process before the drying bed.

With additional controls for the age of the sludge, season of collection and type of digestion, the percent moisture content of the drying bed sludge was not found to be significant with respect to densities viable *Ascaris* eggs, although it was significant for densities *Toxocara* eggs ($p < 0.001$). Fewer viable *Toxocara* eggs were found with decreasing sludge moisture content. The age of the sludge in the drying bed was not found to have a significant effect for either *Ascaris* ($0.25 > p > 0.10$) or *Toxocara* ($p > 0.25$) eggs. The type of digestion process was not significant with respect to *Ascaris* ($p > 0.25$) but was significant with respect to *Toxocara* eggs ($0.05 > p > 0.01$) with fewer viable eggs in the drying bed associated with anaerobic processes than with aerobic. Significantly, fewer *Ascaris* eggs were found in fall than in winter ($0.05 > p > 0.01$), while no seasonal differences were found for *Toxocara* eggs. Well over half of all simultaneously collected samples showed more than 75% fewer viable *Ascaris*, *T. trichiura*, *T. vulpis* and *Toxocara* eggs in drying bed samples than in digester samples. Only five samples from the northern drying beds had a moisture content of less than 20%, which was previously reported to be necessary for effective parasite egg inactivation. Three of these samples were from covered drying beds; while the other two samples were from plants in eastern Washington.

Higher densities of viable *Ascaris* eggs in undigested sludges were significantly associated with higher densities of viable *Ascaris* eggs in drying bed sludges ($p = 0.001$). This was also true for the eggs of *T. trichiura* ($p < 0.03$) and *T. vulpis* ($p < 0.03$). The densities of viable *Toxocara* eggs did not show a significant relationship, however.

Step-up multiple regression analysis was utilized to test the significance of the effect of 1) percent moisture content of the drying bed sludge, 2) age of the sludge in the bed, and 3) digestion process (aerobic vs. anaerobic) employed on the undigested sludge on the inactivation of parasites. No trend was noted for sludge age and the digestion process, but decreasing moisture content of the drying bed sludge was significantly associated with decreased densities of viable *Ascaris* ($0.01 > p > 0.001$), *T. trichiura* ($0.05 > p > 0.01$), *T. vulpis* ($p < 0.001$) and *Toxocara* ($p < 0.001$) eggs.

Sludges Destined for Ultimate Disposal

The type of sludge destined for disposal was dewatered sludge in 31 plants, liquid sludge (primary or digested) in 13 plants, and both dewatered and liquid sludge in 4 plants. In the majority of cases, the sludge was land-applied with only about one-third being placed in landfills, lagoon storage, or the ocean.

The percentages of the samples of these final sludges that contained viable eggs of *Ascaris*, *T. trichiura*, *T. vulpis* or *Toxocara* and the geometric mean of these eggs are presented by state in Table 4. In samples from all four states, *Toxocara* eggs were found most frequently (55%), with *Ascaris* eggs, *T. vulpis* eggs and *T. trichiura* eggs being found somewhat less frequently, 48%, 38% and 22%, respectively. *Ascaris* eggs occurred in higher levels in final sludges than the other three parasites, and the densities of *Ascaris* eggs in final sludges in Washington were significantly higher than in these sludges in the other three states.

The number of plants that distributed sludge to the public for use at home included five in Ohio, four in New York, two in Washington, and one in Minnesota. Viable eggs of *Ascaris*, *T. trichiura*, *T. vulpis* and *Toxocara* were found in 39%, 6%, 56% and 50%, respectively, of the samples of these sludges. The geometric means of viable eggs of each of these parasites found in positive samples were 492, 92, 300 and 580 eggs/kg dry weight, respectively.

Laboratory Studies

Continuous aerobic digestion at 25°C with detention times of 10, 20 and 30 days did not noticeably affect the viability of *Ascaris* eggs in the sludge. However, when digestion was carried out at 35°C, a 30-50 percent inactivation of the eggs was noted within 10 days.

The addition of caustic (NaOH) to sludge previously digested at 25°C had little effect on the viability of the *Ascaris* eggs, even over a period of 10 days. However, when caustic was added to sludge from the 35°C digester, 97% of the eggs were killed within 5 days and 100% were killed within 10 days. In lime stabilization studies, no effect on the *Ascaris* eggs was noted until a lime dosage of 1,000 mg lime per gram of sludge solids was used. At that dosage, 97% of the eggs were killed within 5 days.

When ammonium sulfate at a dosage of 50 mg ammonia per gram of sludge solids was added to sludge previously aerobically digested at 25°C for 10 days, there was little effect on the *Ascaris* eggs during the first five days. After 10 days, 62% of the eggs were inactivated. When the ammonia dosage was increased to 500 mg/gram solids, complete or near complete inactivation was observed after 10 days.

When ammonia gas was added to sludges previously aerobically digested at 25°C at detention times of 10, 20 or 30 days, a dosage of one percent was necessary to obtain effective inactivation of the *Ascaris* eggs. When ammonia was added to sludges digested at 35°C, a higher degree of inactivation was found to occur than with comparable dosages in sludges digested at 25°C.

The exposure of *Ascaris* eggs to an ultrasonic field at 33 KHz and 600 watts for 11 minutes inactivated 77 percent of the eggs.

In studies using gamma radiation from a Cobalt 60 source, it was found that a dosage of 200 Krads or greater was completely effective in killing *Ascaris* eggs in sludge (3% solids). Eggs from the feces of pigs and from the uteri of worms were equally susceptible to the radiation, as were the intact and decoated eggs.

Table 4. *Percentage of Sludge Samples Destined for Disposal in Each of Four Northern States that Contain Viable Eggs of* Ascaris, Trichuris trichiura, T. vulpis *and* Toxocara *and Mean Number of Eggs of Each Parasite*

Eggs	*MN* (STATE)	*OH*	*NY*	*WA*	*All Samples*
Ascaris					
% positive	*49%*	*41%*	*49%*	*47%*	*48%*
Geometric Mean[1]	*375*	*235*	*560*	*1920*	*565*
T. trichiura					
% positive	*14%*	*12%*	*31%*	*33%*	*22%*
Geometric Mean	*140*	*150*	*390*	*280*	*265*
T. vulpis					
% positive	*12%*	*63%*	*56%*	*28%*	*38%*
Geometric Mean	*255*	*270*	*230*	*395*	*270*
Toxocara					
% positive	*51%*	*68%*	*49%*	*50%*	*55%*
Geometric Mean	*315*	*280*	*455*	*515*	*370*

[1]*Antilog of the mean of the log of positive samples; expressed as number of eggs/kg dry weight of sludge.*

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The complete report, entitled "Investigation of Parasites in Sludges and Disinfection Techniques," (Order No. PB 86-135 407/AS; Cost: $22.95, subject to change) will be available only from:
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