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Project Summary

Helminth and Heavy Metals Transmission From Anaerobically Digested Sewage Sludge

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This summary discusses the findings of a study designed to determine the transmission to an animal host of the ova of the nematode worm *Ascaris* sp. that have survived through a modern sewage treatment process and are present in the sludge. Four large experiments and three smaller ones involving 178 specific pathogen-free (SPF) pigs were used. Natural transmission of *Acaris* sp. from soil treated with liquid anaerobically digested sewage sludge that had been stored for several years occurred in a few pigs in each of four experiments. Also, natural transmission from Nu-Earth, a dried, stored sewage sludge, also occurred in pigs that were exposed to this material by contact in the pens. In general, ova in anaerobically digested sludge or in Nu-Earth remained unembryonated until after they were exposed to the air. Within 6 weeks after exposure to air, the ova began to embryonate, and thereafter, a small percentage of the ova that embryonated became infective for pigs.

The occurrence of heavy metals in the tissues of swine held in pens treated with anaerobically digested sludge or Nu-Earth, which originated from a large municipality, was also studied. Chemical analyses of kidneys, livers, hearts, diaphragm muscles, and bones were conducted to determine the quantities of the heavy metals cadmium, zinc, copper, iron, lead, chromium, and nickel that were present in the tissues following exposure of the pigs to different amounts of the sewage products in or on the soil. Only cadmium accumulated to a significant degree in some tissues of swine exposed to sludge containing heavy metals. No physiological or pathological changes associated with exposure to the sludge material were detected. Examination of visceral fat from control and experimental pigs indicated that there was no unusual accumulation of organic compounds including polychlorinated biphenyls (PCB's) and the insecticides Heptachlor and Dieldrin.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, 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

Improvements in sewage disposal systems within the last 50 years have contributed to improved use of biological wastes to supplement the texture and fertility of soils. In using aerobically or anaerobically digested sewage sludges for soil conditioning or fertilizer, one of the important unanswered questions, however, concerns possible adverse

effects of dispensing of possible disease-causing materials to the environment.

Farmers throughout the world have used human and animal fecal material as fertilizer for hundreds of years. Only since the early 1900's has the use of such materials been recognized as the source of many diseases. More recently, it has been determined that a few bacterial, viral, protozoan, and helminth parasites are able to persist in waste products originating from humans or animals. Although the majority of pathogenic organisms of viral, bacterial, protozoan, or helminth origin are destroyed by exposure to an external environment, many validated instances of serious outbreak of disease have occurred involving these organisms. In underdeveloped countries, many parasitic diseases are literally a "way-of-life."

During recent years, researchers have found that ova of ascarid roundworms are the most resistant organisms in wastes from human and animal sources. These ova are known to survive in soil for many months, even years, and thus serve as a potential source of disease.

In addition to the bacteria, viruses, protozoan, helminths, etc., other materials found in sludges may cause disease in man and animals. Among these are toxic chemicals such as heavy metals (e.g., Cd, Pb, Hg, Cr, etc.) and organic compounds (e.g, PCB's) of various kinds and combinations. The route of these chemicals to man or animals is through direct ingestion of sludge, or through the food chain that starts with crops grown on sludge-amended soil. The relationship between exposure or dose and illness is complex and is not the subject of this investigation However, the chance of illness is clearly related to the flow of the toxic substance up the food chain. By establishing relationships between the initial dose or exposure and the absolute concentrations in the intermediate and final hosts in the food chain, the ultimate threat of illness can be put in perspective.

In the work summarized here, one objective was to determine the possibility of transmission of one pathogen (*Ascaris lumbricoides suum*), a resistant parasite, from soil treated with sludge to a susceptible host (a pig). This parasite is almost identical in nature and in its mode of infection to the *Ascaris lumbricoides*, which infects man. Consequently, information developed on transmission bears directly on the dose-infection relationship for pigs and is most likely very similar to the dose-infection relationship in humans. A second objective was to determine the uptake of heavy metals and organic compounds in the pigs exposed to sludge. From this information, the possible exposure of humans who ultimately consume the pigs can be estimated.

Procedure

A total of 178 pigs free from parasitic infections (specific-pathogen-free:SPF) were used to study whether anaerobically digested sludge, added to soil at different concentration levels, could be a potential source of infestation with the nematode *Ascaris lumbricoides suum*. The soil was treated with various levels of liquid anaerobically digested sludges (from 22 to 65 dry metric tons per hectare) or with dried sludge (Nu-Earth) added to the soil to a depth of 15.3 cm. Pigs were confined in pens (9.8 x 14.6 m) to which the sludge had been added. Pigs living in each of these environments, for approximately 4 months, were compared with pigs living in similar control pens, under the same environmental conditions, but without sludge added to the pens.

Results

- In each of two of the experiments, eight young pigs were necropsied 2 to 3 weeks after introduction into the pens and lung and liver tissues were Baermannized to recover migrating stages of the worms. Larvae were recovered from the lungs of 2 of 16 necropsied. Of 134 pigs that survived the full experiment to necropsy, 43 contained ascarid worms in the intestines, including four from the control pens. These four represent a contamination of the control area that apparently occurred accidentally during the course of the study. These results represent an infection rate of 28% in the experimental groups and 3% in the control groups. Of the 134 pigs, 11 were housed in pens treated with previously stored Nu-Earth; of these, 6 were infected.
- An attempt was made to determine the rate of transmission of the human ascarid worm, obtained from a source in Columbia, South America, to pigs. Fifteen SPF pigs were confined in two sludge-free pens. Embryonated or unembryonated ova in water were sprayed onto the soil or vegetation at the rate of 572,000 ova in each of the two pens. The ova were extracted from the uteri of adult female human *Ascaris lumbricoides;* the portion of embryonated were embryonated in 1% formalin with continuous aeration for 30 days. The pigs were allowed to remain in the environment for 4 months, During that time, all of the vegetation was consumed and the pigs did what pigs do: ate soil, breathed dust, rolled in water and mudholes after rainstorms, etc. At necropsy, 4 months after being placed in the pens, no worms were found in either group of pigs.
- Three pigs were fed anaerobically digested sludge in which there were naturally occurring ascarid ova as part of their normal food diet. The source of the ascarid ova, of course, could have been humans or pigs. Before the feeding step, the liquid anaerobically digested sludge was aerated by bubbling air through it for 104 days. It was then fed to each of the three pigs during a 30 day period. Forty-seven days after the end of the feeding period, the pigs were necropsied and the gastrointestinal tracts were examined for ascarid worms. Worms found in the intestine of one pig indicated that ova naturally present in the sludge could be infective for pigs.
- The rate of embryonation of ascarid ova (removed from the uteri of gravid female worms) was determined in the presence of untreated soil, soil treated with liquid anaerobically digested sludge, or Nu-Earth. At the end of 6 weeks' exposure to the soil to which liquid sludge or Nu-Earth had been added, an average 71% of the ova had embryonated and were presumed to be infective. The worms were determined to be viable by observation of movement of larval stages within the ova. None of the ova were fed to susceptible pigs to ascertain infectivity.
- Tissues taken from the pigs at necropsy were analyzed for heavy metals and organic compounds. Diaphragm muscle, heart, liver, kidney, and bone were selected for analysis for heavy metals, and visceral fat

was selected for analysis for the organic compounds (Tables 1 and 2).

Cadmium in the kidney and liver was the only heavy metal showing significant accumulation. Some heavy metals present in sludges may accumulate in the tissues at levels related to the level of exposure, i.e., animals exposed to greater concentrations of sludge had greater tissue concentration of some heavy metals. In these studies, pigs exposed to dried sludge (Nu-Earth) accumulated the greatest quantities of heavy metals. For example, the average accumulation of cadmium in the kidneys was 9.8 ppm dry weight. On a wet weight basis this would be less than 3 ppm. In humans, clinical evidence of damage to the kidney is said to begin when approximately 200 ppm, wet weight, have accumulated.

The results of chemical analysis for organic compounds (PCB's, Dieldrin, Heptachlor) showed there was no difference between experimental and control animals in terms of accumulation. These compounds were not accumulated in the animal tissues to levels greater than is normally found in the environment.

Conclusions

Exposure of swine to different levels of anaerobically digested sludge from a large municipality has shown that a potential for transmission of the nematode *Ascaris* sp. exists. The results of this study show that some ova from this parasite worm are able to withstand the rigorous treatment of a modern sewage treatment plant and are subsequently infective to pigs. The exposure of swine to anaerobically digested sludge was infinitely greater than a normal human exposure would be, yet the levels of infestation with *Ascaris* sp. were low. With ova present at an approximate level of 4000/m^2 of surface, embryonation of some ova occurred, some were ingested by pigs and eventually caused light infestations in the gastrointestinal tracts. Although these worms did not cause detectable pathology in the animals they infested, a potentially serious parasite problem could develop in a swine herd continuously exposed to anaerobically digested sludge. This could occur not because of the immediate damage caused by worms transmitted from the sludge, but because of the potential for buildup of numbers of ova in the environment as sexually mature female worms, originating from ova in the sludge, began to lay eggs which would "seed" the environment. Subsequent swine, exposed to the area, could be exposed to much greater numbers of ova, and a potential parasite problem could develop into a serious actual problem.

Under the conditions of this study, the health of the animals did not appear to be threatened by the parasites transmit-

Table I. *Summary of chemical analyses for heavy metals in tissues of pigs exposed to different levels of anaerobically digested sewage sludge originating from the Metropolitan Sanitary District of Greater Chicago. Numbers in parenthesis represent deviations in numbers of animals.*

		ppm, dry weight				
Metal & Group	Number of Animals	Kidney	Liver	Diaphragm Muscle	Heart	Bone
Cadmium						
Control	14	0.47	0.23	0.04	0.05	0.03[2]
22-25 DMT/Ha sludge	18	2.30	0.34	0.06	0.05	0.02[6]
33 DMT/Ha sludge	4	2.10	0.29	0.07	0.22	—
65 DMT/Ha sludge	4	3.83	0.58	0.15	0.09	—
Nu-Earth	4	9.80	1.50	0.06	0.06	0.04
NBS control (bovine)			0.27			
Chromium						
Control	12			0.12	0.08	
22-25 DMT/Ha sludge	12			0.17	0.09	
33 DMT/Ha sludge	4			0.14	0.18	
65 DMT/Ha sludge	4			0.09	0.06	
Nu-Earth	—			—	—	
Copper						
Control	14	37.00	26.00	7.00	16.00	3.50[2]
22-25 DMT/Ha sludge	18	33.00	25.00	10.00	17.00	3.30[6]
33 DMT/Ha sludge	4	28.00	37.00	9.00	19.00	—
65 DMT/Ha sludge	4	33.00	23.00	8.00	16.00	—
Nu-Earth	4	44.00	33.00	7.00	18.00	3.80
NBS control (bovine)			188.00			
Iron						
Control	14	166.00	488.00	99.00	180.00	44.00[2]
22-25 DMT/Ha sludge	18	155.00	541.00	103.00	187.00	44.00[6]
33 DMT/Ha sludge	4	144.00	549.00	91.00	169.00	—
65 DMT/Ha sludge	4	172.00	586.00	119.00	180.00	—
Nu-Earth	4	198.00	453.00	73.00	152.00	42.00
Lead						
Control	14	0.31	0.33	0.22	0.20	0.63[2]
22-25 DMT/Ha sludge	18	0.31	0.34	0.28	0.23	0.69[6]
33 DMT/Ha sludge	4	0.29	0.36	0.17	0.18	—
65 DMT/Ha sludge	4	0.32	0.31	0.20	0.38	—
Nu-Earth	4	0.44	0.47	0.21	0.26	1.77
NBS control (bovine)			0.35			
Nickel						
Control	12			0.39		
22-25 DMT/Ha sludge	12			0.41		
33 DMT/Ha sludge	4			0.30		
65 DMT/Ha sludge	4			0.60		
Nu-Earth	—			—		
Zinc						
Control	14	138.00	264.00	123.00[9]	75.00	98.00[2]
22-25 DMT/Ha sludge	18	133.00	263.00	128.00[11]	74.00	118.00[6]
33 DMT/Ha sludge	4	117.00	248.00	119.00	65.00	—
65 DMT/Ha sludge	4	128.00	210.00	108.00	74.00	—
Nu-Earth	4	140.00	286.00	113.00	77.00	122.00
NBS control (bovine)			131.00			

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Table 2. *Summary of chemical analyses for organic compounds in visceral fat of pigs exposed to different levels of anaerobically digested sludge originating from the Metropolitan Sanitary District of Greater Chicago.*

Exposure	Number of Pigs	ppm, wet weight: Polychlorinated Biphenyls	Heptachlor Epoxide	Dieldrin
Control	17	0.158	0.063	0.051
22 DMT/Ha sludge	9	0.111	0.005	0.006
Nu-Earth	10	0.120	0.002	0.003

ted through the sludge. If humans were exposed to fields with the same sludge application densities, they would ingest far less sludge than did the pigs. Consequently, it is not likely that man would become seriously parasitized from exposure to anaerobically digested sludge under current sanitary conditions in the United States.

The results of this study have shown that cadmium was the only one of seven heavy metals which accumulated to a significant degree in tissues of swine exposed to sludge containing heavy metals. Of the five tissues examined for heavy metals, only kidney and liver accumulated cadmium at levels greater than in controls. Although the accumulation in these organs was statistically greater than in controls, the accumulation was far below a lethal level.

The presence of polychlorinated biphenyls and the organic insecticides Heptachlor and Dieldrin in tissues of experimental animals, at levels comparable to controls, indicates that these organic compounds did not appear to be an immediate threat to animal health under the conditions of these studies. The facts revealed herein suggest that serious exposure to anaerobically digested sludge, containing organic compounds at levels described in this study, would cause no significant detrimental effect on the animals.

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***Gerald Stern** is the EPA Project Officer (see below).*
The complete report, entitled "Helminth and Heavy Metals Transmission from Anaerobically Digested Sewage Sludge," (Order No. PB 81-161 846; Cost: $8.00, subject to change) will be available only from:
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