

Survival Rates of Parasite Eggs in Sludge During Aerobic and Anaerobic Digestion

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The effects of mesothermic anaerobic or aerobic sludge digestion on survival of eggs from the roundworms *Ascaris suum*, *Toxocara canis*, *Trichuris vulpis*, and *Trichuris suis* and from the rat tapeworm *Hymenolepis diminuta* were studied. Destruction of eggs throughout a 15-day treatment period, as well as their viabilities after reisolation, was analyzed. The laboratory model digesters used in this study were maintained at a 15-day retention schedule, partially simulating a continuously operating system. *Ascaris* eggs were destroyed in the anaerobic (23%) or aerobic (38%) digesters, and 11% *Trichuris* eggs were destroyed in the aerobic digesters. *Trichuris* eggs in anaerobic digesters and *Toxocara* eggs in either anaerobic or aerobic digesters were not destroyed. Destruction of eggs in digesters was correlated with the state of the eggs before subjection to the treatment processes; i.e., some *Ascaris* and *Trichuris* eggs were already embryonated in host intestinal contents or feces and hence past their most resistant stage. The viabilities of *Ascaris* and *Toxocara* eggs that survived the digestion processes were greater in anaerobically treated than in aerobically treated material. Eggs from *Hymenolepis* were nonviable before use in the experiments. However, they were more effectively destroyed in aerobic digesters than in anaerobic digesters.

A steady growth in urban areas brings with it the problem of disposal of the increased products of municipal sewage treatment. Today, most treatment of raw sewage consists of settling and activated sludge, sometimes followed by anaerobic or aerobic digestion. These processes serve to concentrate solids and, concomitantly, parasite eggs. Some parasites, e.g., *Trichinella*, *Eimeria*, *Toxoplasma*, *Entamoeba*, and perhaps *Giardia* and *Schistosoma*, are eliminated by these processes (6, 8, 9), but there are others that are resistant. Eggs of the enteric parasites *Ascaris*, *Toxocara*, *Toxascaris*, and *Trichuris* have been shown to pass through digesters and remain viable and capable of infection (7, 18, 20). These parasite eggs are commonly found in sludge samples from various countries, including the United States (12, 20). Fitzgerald and Ashley (7) have demonstrated that 77% of *Ascaris* eggs kept in sludge at 38°C for 25 days later embryonated and were infective. Hence, if sludges containing these pathogens are used in topical soil applications, there is a danger of transmittance of disease and parasite infection. *Ascaris* eggs have been able to survive in soil for at least 7 years; tubercle bacilli, up to 6 months; *Salmonella*, up to 112 days; and cysts

of *Entamoeba*, for 8 days (3, 17, 19).

The present study on the rate of parasite egg survival during mesothermic anaerobic or aerobic digestion was conducted as part of a larger project investigating the rate of parasite egg destruction upon storage. This report represents the first study that includes the effects of both aerobic and anaerobic digestions of eggs of three species of roundworms commonly found in sewage, as well as eggs of a tapeworm for purposes of comparison. This was an attempt to simulate authentic high-rate municipal digesters with constant removals and additions (as contrasted to digestion of batch samples of sludge) under controlled laboratory conditions. Furthermore, we addressed the question of the effects of the digestions on the quantitative recoveries of eggs compared with effects of physical entrapment of eggs in sludge alone. The viabilities of eggs recovered were also analyzed.

MATERIALS AND METHODS

Organisms. *Toxocara canis* and *Trichuris vulpis* eggs were recovered from feces of naturally infected dogs obtained from the Society for the Prevention of Cruelty to Animals (Cincinnati, Ohio) and housed in the Lab of Animal Medicine (University of Cincinnati,

Cincinnati, Ohio) or from feces from naturally infected dogs cared for by the Findlay Humane Society (Findlay, Ohio). *Ascaris suum* and *Trichuris suis* eggs were isolated from intestinal contents of naturally infected pigs obtained from the Pioneer Packing Plant (Bowling Green, Ohio) and the Dinner Bell Packing Plant (Archbold, Ohio). *Hymenolepis diminuta* eggs were isolated from feces of rats infected by feeding them adult *Tenebrio* beetles containing cysticercoids (Carolina Biological Supply Co., Burlington, N.C.).

Isolation of eggs from fecal material was according to the following protocol. Approximately 100 g of feces was soaked in 1 liter of water for 10 to 12 h (11) and then passed through a U.S.A. standard testing sieve no. 10. The filtrate, containing eggs, was allowed to stand for 1 h, and then the supernatant was decanted. The sediment was then sequentially processed through sieves no. 18, 25, 35, 60, 100, and 150. After the final sediment was washed four times with water, 30 ml was placed in 250-ml polyethylene centrifuge bottles. The bottles were filled with 71% sucrose and capped, and the contents were mixed. The mixture was centrifuged at $100 \times g$ for 5 min at 4°C in a swinging-bucket rotor (IEC PR-2 preparative centrifuge; I.E.C., Needham Heights, Mass.). The eggs at the top of the liquid were removed and washed four times with water in conical tubes by centrifugation at $550 \times g$ for 2 min. Eggs were isolated, accumulated, and stored at 4°C over a period of 5 months. The two species of *Trichuris*, *T. suis* and *T. vulpis*, were not further distinguished in this study.

During collection of eggs, it was noted that ca. 5 to 10% of *Ascaris* eggs and 1 to 5% of *Trichuris* eggs, but no *Toxocara* eggs, were already embryonated. Therefore, it was assumed that some *Ascaris* and *Trichuris* eggs apparently had passed the most resistant phase of their life cycle within intestinal contents or feces of the host.

Anaerobic digesters. Bench top model anaerobic sludge digesters were constructed from 20-liter Lucite cylinders by I. Kugelman (U.S. Environmental Protection Agency), and these were kept in a room maintained at 35°C. Each digester was started with 4 liters of anaerobic and 11 liters of primary sludge from the Sycamore Sewage Treatment Plant (Cincinnati, Ohio). The feed for the digesters was primary sludge from the Muddy Creek Sewage Treatment Plant (Metropolitan Sewage District, Cincinnati, Ohio). Both plants treat virtually all domestic sewage, but the Sycamore plant has anaerobic treatment facilities whereas the Muddy Creek plant does not. The feed was diluted with water when necessary or thickened with gravity-thickened primary sludge to ca. 5% total solids. The feed was warmed to 35°C before being added to the digesters.

Digester stabilization was accomplished with an initial feed of 500 ml/day for 1 day and eventually maintained at a 15-day retention time by increasing the feed increments by 100 ml/day up to a feed rate of 1 liter/day. The units were maintained at a constant sampling and feed rate for 30 days to achieve equilibrium before parasite eggs were added. The digesters were sealed and mixed for 5 min/h by pumping the enclosed gases through the sludge. The pumps operated continuously during the period of daily withdrawals and additions to ensure homogeneous distributions of digester contents.

The following criteria were used to determine stabil-

ity in the digesters: (i) alkalinity, 2 to 3 g/liter as CaCO_3 (potentiometric analyses) and 1.5 to 2.0 g/liter as CaCO_3 (methyl purple analyses); (ii) pH 7.15; (iii) chemical oxygen demand, 54.9% reduction; (iv) gas composition—50% methane, 35% CO_2 , 1.6% O_2 , 12.5% N_2 ; (v) gas volume, 14 to 22 liter³/day; (vi) total Kjeldhal N (organic), 1.05 g/liter, and ammonia N, 0.68 g/liter; (vii) 4.8% total sludge feed solids, 33.6% reduction of total solids, and 23.8% reduction of volatile solids; and (viii) volatile acids, ca. 0.3 g/liter as acetic acid. Parameters tested demonstrated that the digesters were operating under stabilized conditions (1, 2, 5, 14, 16).

The parasite eggs were added to the digesters by pouring the eggs into the inlet funnel followed by the feed sludge. Additions were made as rapidly as possible and only after the inlet funnel was filled to minimize the introduction of air into the system. The digesters were mixed for 30 to 40 min to ensure homogeneous distributions of eggs in the units.

Aerobic digesters. Lucite cylinders for the aerobic digesters were similar to those used for the anaerobic digesters. These units were maintained in a 20°C constant-temperature room. The digesters were open to the atmosphere and were aerated and mixed with in-house compressed air that delivered a minimum flow of 0.015 ft³ (ca. 0.0004 m³)/min. Each digester was filled with 15 liters of activated sludge from the Muddy Creek Treatment Plant and operated at a 15-day retention time, using the protocol of the anaerobic digesters described above. The feed was obtained from secondary sludge from the Muddy Creek plant (excess return), which was thickened to about 3% total solids by adding gravity-thickened sludge (mostly primary). The following criteria were used to indicate stabilization of organic matter in the aerobic digesters: (i) alkalinity, 0.34 g/liter as CaCO_3 ; (ii) pH 7.29; (iii) chemical oxygen demand reduction, 37%; (iv) suspended solids reduction, 15.3%; (v) reduction of volatile suspended solids, 16.4%; and (vi) total volatile solids reduction, 14.1% of total solids (2, 13, 16).

Addition of eggs to digesters. All sludge material used for feeds and initiation of anaerobic and aerobic digesters was devoid of parasite eggs. After the digesters were stabilized, daily additions of sludge with the parasite eggs were made. On the last day (day 16) of these treatments, all anaerobic digester contents and all aerobic digester contents were pooled. These were mixed with a 8-mm-diameter multi-hairpin-looped metal rod while 100-g aliquots were prepared. Recoverable eggs (see below) from these samples were not different from those in the daily sampling during the digestion process, indicating that there was a homogeneous distribution of eggs during the operation of the digesters.

Addition of eggs to digested sludges. To test the effectiveness of the digestion process, control samples were prepared. Egg-free stabilized anaerobically and aerobically digested sludge samples from different times during the 15-day retention time were seeded with eggs after the digestion treatment. Eggs were isolated from these samples by the same techniques described for egg recoveries below. The recovery rates obtained from these samples for each parasite species were designated as 100% controls and represent the recoverabilities of these various eggs. These control values account for entrapment of eggs to solids

TABLE 1. Number of parasite eggs added to each 15-liter anaerobic or aerobic digester

Eggs	Initial (day 1) addition (total eggs/digester)	Daily additions (total eggs) ^a
<i>Ascaris suum</i>	1.5×10^6	1.0×10^5
<i>Toxocara canis</i>	3.1×10^5	2.1×10^4
<i>Trichuris vulpis</i> and <i>T. suis</i>	1.4×10^5	9.3×10^3
<i>Hymenolepis diminuta</i>	1.9×10^6	1.3×10^5

^a Each day, 1 liter of digester contents was removed and 1 liter of sludge feed was added with the parasite eggs.

in the sludges and other physiochemical parameters that influence recovery rates; these eggs were not treated in any digester.

Recovery of eggs from sludge. Sludge samples (100 g) were filtered through a U.S.A. standard testing sieve no. 35 and washed with water. A 30-ml portion of the filtrate was added to 20 ml of 3% beef extract (Oxoid U.S.A. Inc., Columbia, Md.) to inhibit adherence of sludge material to eggs. The mixture was centrifuged at $400 \times g$ for 5 min at 4°C. The supernatant was decanted, and the pellet was washed free of beef extract with water. This pellet was suspended in water, and 10 ml of the suspension was layered on a 30 to 35-ml continuous concave sucrose density gradient and centrifuged at $1,100 \times g$ for 10 min. The pellet was discarded, and material in the gradient was collected, diluted with an equal volume of water, and centrifuged at $800 \times g$ for 5 min. All pelletable material from a single 100-g sample was pooled, centrifuged, and reduced to 2 ml. The material was evenly resuspended and 1 ml was analyzed for numbers of eggs (by species), using a Sedgewick-Rafter counting chamber and a Whipple ocular disk.

Viability. After the eggs were isolated from the sludge they were placed in a plastic petri dish (100 by 15 mm) and incubated in ca. 10 ml of water with 0.1 ml of an antibiotic-antimycotic mixture (GIBCO Laboratories, Grand Island, N.Y.) at 25°C in the dark. After 30 days, the samples were examined for total egg numbers and those that were embryonated (10). The percentage of eggs embryonated was designated as percent viable eggs. An egg was considered embryonated if a worm was visible within the egg shell. The morphological changes appearing in eggs that were used as criteria of dead eggs were cytolysis of egg cell(s) (after cytokinesis had begun), formation of large refractile granules within the cell, vacuolation or hyalinization in the cytoplasm, shrinkage of the egg, disintegration of the membrane surrounding the egg cell, and collapse of the egg shell. Viability was distinguished from infectivity, which was not tested in this particular phase of the study.

RESULTS

Addition of eggs to sludge. Eggs of various parasites were quantified and added to digesters at the beginning of the 15-day digestion period and with daily feeds (Table 1). Control samples

were prepared by adding parasite eggs to digested sludge, and the percent eggs recovered from these samples was determined (Table 2).

Survival of parasite eggs during sludge digestion. Recoveries of *Ascaris* eggs from anaerobically and aerobically digested sludges were consistently below those of the control values, indicating that both of these digestion processes destroyed these eggs (Fig. 1A). A nonparametric sign test (22) indicated that the digestion and control recovery values in both anaerobic and aerobic experiments were significantly different ($P < 0.01$). The mean egg destruction by anaerobic digestion of *Ascaris* eggs was 23%, and that by aerobic digestion was 38%. Recoveries of *Toxocara* eggs were not different from control values under the two digestion conditions (Fig. 1B). Hence, the eggs of this species were apparently resistant to both anaerobic and aerobic digestion processes. *Trichuris* eggs were not destroyed by anaerobic digestion; however, destruction of 11% of these eggs ($P < 0.01$) occurred by aerobic digestion (Fig. 1C). Therefore, some *Ascaris* eggs were destroyed during both anaerobic and aerobic digestions, *Trichuris* eggs were destroyed only during aerobic digestion, and *Toxocara* eggs were not destroyed during either anaerobic or aerobic digestion.

Viability of recovered parasite eggs. Of those eggs recovered from the sludge samples (designated as 100%), the percent that embryonated after incubation was calculated. This value was taken as the percent viability of recovered eggs (Fig. 2). The viabilities of all parasite eggs recovered during anaerobic digestion decreased during day 5 of the treatment protocol. Eggs of all species were affected, indicating that a general deleterious condition occurred on that day. With time, the percent viability of recovered eggs increased to original levels. Another decrease in the percent viability of recovered *Trichuris* eggs at the end of the treatment period (days 15 and 16), which was not observed in the viabilities of *Ascaris* or *Toxocara* eggs recovered during the same period, remains unexplained. The results

TABLE 2. Recoverability of parasite eggs from digested sludge (controls)

Species	% Recovered ^a	
	Anaerobic	Aerobic
<i>Ascaris suum</i>	75 ± 23	80 ± 10
<i>Toxocara canis</i>	63 ± 1	58 ± 9
<i>Trichuris vulpis</i> and <i>T. suis</i>	83 ± 18	35 ± 4

^a A total of 100 eggs of each species were added to 10 ml of sludge and reisolated. Data are expressed as the means of six anaerobic and five aerobic samples ± standard deviation.

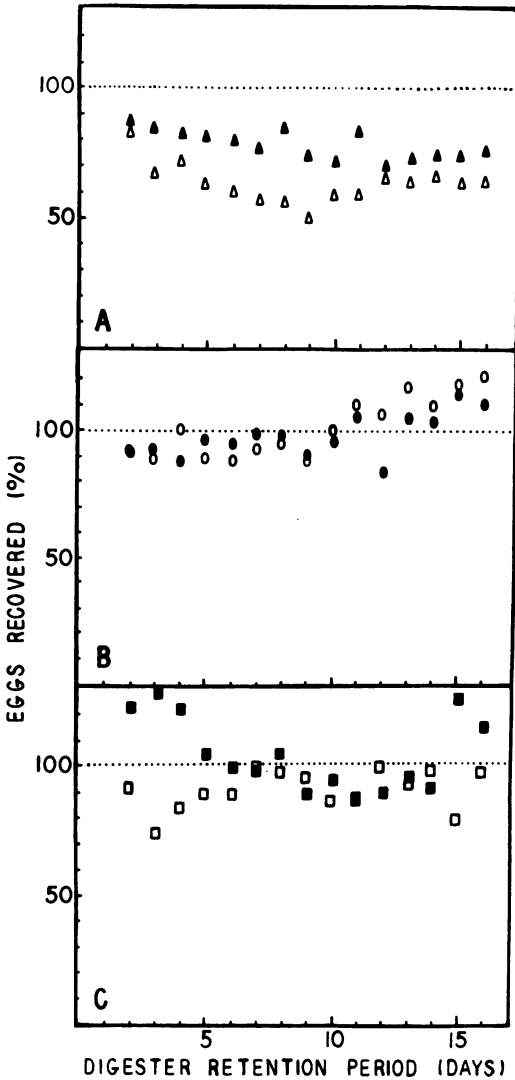


FIG. 1. Survival of *Ascaris* (A), *Toxocara* (B), and *Trichuris* (C) eggs during anaerobic (closed symbols) or aerobic (open symbols) digestion. The dotted horizontal lines at 100% represent the normalized control values for recoverability of eggs, i.e., percent eggs recovered from samples of digested sludges to which untreated (undigested) eggs were added. Control values are the means of six (anaerobic) and five (aerobic) determinations. Each point represents the means of three determinations. (A) Ranges, 1 to 16% (anaerobic) and 2 to 29% (aerobic); (B) ranges, 4 to 29% (anaerobic) and 6 to 28% (aerobic); (C) ranges, 6 to 60% (anaerobic) and 3 to 20% (aerobic).

on *Trichuris*, however, suggest that about 70% of those eggs recovered remained viable.

Results on the viabilities of eggs recovered from sludge during aerobic digestion were more definitive than those on anaerobic digestion in

this study. Approximately 90% of *Toxocara* and *Ascaris* eggs recovered from sludge from the aerobic digesters remained viable throughout the treatment period. *Trichuris* eggs exhibited a progressive decline in viability with treatment time, and about 85% of the eggs were rendered nonviable near the 15-day retention time of the digesters.

H. diminuta. Unlike the nematode eggs described above, the eggs of the rat tapeworm *H. diminuta* were nonviable before being added to the digesters. Storage over several months during the accumulation of eggs, even at 4°C, failed to preserve their viabilities. These eggs were nonetheless tested for destruction in anaerobic and aerobic digestions. The results suggest that aerobic digestion was more effective than anaerobic digestion in destroying these eggs (Fig. 3). These eggs appeared to decrease in numbers in the anaerobic digesters with time of treatment.

DISCUSSION

We have demonstrated the effects of mesothermic anaerobic and aerobic digestion pro-

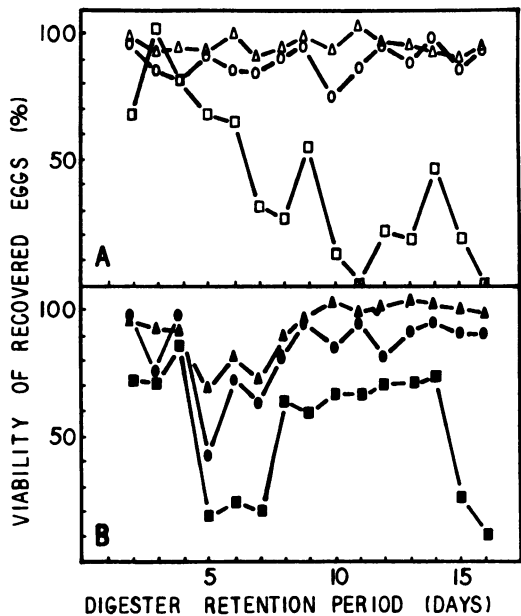


FIG. 2. Viability of eggs recovered during aerobic (A) and anaerobic (B) digestion. Of the eggs recovered from samples withdrawn from the digesters during the 15-day experiment, the percent that embryonated after an incubation period was designated as the percent viability. (A) *Toxocara* (○) and *Ascaris* (△) eggs that were not destroyed in the aerobic digesters remained viable. In contrast, aerobic digestion progressively and effectively reduced the viability of *Trichuris* (□) eggs. (B) *Toxocara* (●) and *Ascaris* (▲) eggs recovered from anaerobic digester samples remained viable, whereas *Trichuris* (■) eggs had reduced viabilities.

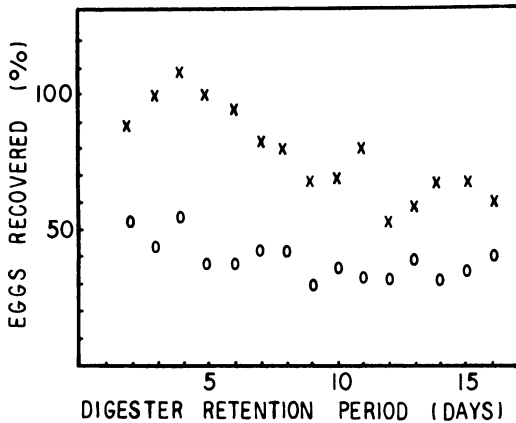


FIG. 3. Recovery of *Hymenolepis* eggs from aerobic (O) and anaerobic (X) digesters.

cesses on the eggs of three roundworms and one tapeworm within sludge material under controlled laboratory conditions. Since the study was to simulate digesters with continuous intake and removal (15-day retention time), the population of eggs in the digesters at the end of the treatment (i.e., day 16 in this study) included those eggs that were there from 1 to 15 days. Conditions at the end of the treatment, therefore, partially simulated steady-state conditions of a continuously operating system, and the destruction and viability of eggs of these samples are what might be expected for eggs in sludge in a continuously operating plant.

The drop in viabilities of all parasite eggs on day 5 in the anaerobic digesters could be explained by the presence of a toxic substance(s) in that day's primary sludge feed. The subsequent increase of viabilities back to the original levels that occurred with continued digester content turnover is consistent with this interpretation.

The reduction of *Ascaris* eggs over control values under both digestion conditions is explained by the observation that a significant percentage of eggs of this species were already embryonated before exposure to the treatment. Unlike other studies in which eggs at different stages of development were removed from adult worms and used for experiments (15), in this study only eggs that were shed by the worms were used. Only shed eggs were used because, unlike immature eggs taken from worm uteri, these eggs would be expected to have their full complement of shell components and to have had appropriate time for physiological processes such as tanning (4) that render them resistant. Also, it is more likely that mature, shed eggs rather than immature eggs would be present in sludge. The finding that some shed eggs were

already embryonated was unexpected and suggests that these and other eggs were past their most resistant stage of their life cycle and therefore susceptible to either digestion process.

No *Toxocara* eggs were embryonated before treatment, and recoveries of these eggs from anaerobically or aerobically digested sludge were not different from controls. The relative greater resistance of *Toxocara* eggs to digestion over that of *Ascaris* eggs was also observed by Riemers et al. (21).

Only a few *Trichuris* eggs were embryonated, and the results indicate that destruction of eggs of this species occurred only in the aerobic digestion process. This difference between the effects of digestion processes was also true of the nonviable *Hymenolepis* eggs; i.e., aerobic digestion was more effective in destroying these eggs than was anaerobic digestion. These observations are unexplained but interesting in that total solids and volatile solids reduction in the anaerobic digesters was greater than in the aerobic digesters. Furthermore, the anaerobic digesters were maintained at a higher temperature (35°C) than the aerobic digesters (20°C). These temperatures were selected for these studies since they represent those temperatures presently used by sewage treatment plants. Several studies indicate that the temperature at which the digesters are maintained is the determining factor in parasite egg destruction, rather than the type of digestion process (21; also see below). Perhaps that may be true of gross temperature comparisons, e.g., 55 versus 20°C (21), but the mesothermic conditions we used may have enabled us to detect more subtle differences on the effects of digestion processes on certain species of parasite eggs.

The viability of eggs isolated from samples throughout the study indicated that those *Ascaris* and *Toxocara* eggs surviving either digestion treatment remained viable. In contrast, the treatments, particularly aerobic digestion, were effective in decreasing the viabilities of *Trichuris* eggs.

Further studies in progress in this laboratory indicate that those eggs not destroyed within the digesters were not further destroyed after several months in storage, they are infectious, and long-term storage of eggs in the sludge (simulating lagooning processes) reduces the viabilities of the eggs with time. The rates of reduction in egg viabilities appears to be closely correlated with the temperature of storage.

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