ORIGINAL RESEARCH

Survival of bacteria and virus in ground piglet carcasses applied to cropland for disposal

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Summary

Objective: *To determine the survival of bacterial and viral organisms in soil to which liquid swine manure containing ground piglet carcasses was applied.*

Design and procedures: *Piglet carcasses up to 5.5 kg (12 lb) were homogenized using a commercial-sized grinder. Liquid swine manure collected from an anaerobic pit was applied to the surface of cropland either with no immediate tillage or by subsurface injection into soil. After manure was applied to control plots, homogenized piglet carcasses containing* Salmonella anatum *(6×10^{11} CFU) and T_1 coliphage (3.1×10^{12} PFU) were added to liquid swine manure before applying them to the surface of cropland either with no immediate tillage or by subsurface injection.*

Results: *Adding ground piglet carcasses had no effect on yield of corn grain.* Salmonella anatum *survived for ≤56 days, and coliphage survived ≤20 days after being applied to soil. The method of manure application had no influence on the survival of* S. anatum. *In contrast, coliphage survived longer when manure was injected compared to applying it to the surface of the soil.*

Implications: *The potential for* S. anatum *and pathogenic viruses to contaminate soil used for row crop production when manure containing ground pig carcasses is applied to the soil appears to be small.*

Keywords: *swine, carcass disposal, pathogens*

Date received: *Jan 3, 1996*
Date accepted: *Apr 19, 1996*

Inherent in any livestock production system is the need to dispose of animals that die or are euthanized before being marketed. Morrow and Ferket[1] estimated that a 1000-sow farrow-to-finish operation would produce about 8000 kg (9 tons) of dead pigs annually. With over five million producing sows in the United States, the number of dead pigs produced presents significant challenges for safe disposal of carcasses. Existing methods of disposal include burial, incineration, rendering, composting, fermentation, disposal pits, and refeeding to other animals such as mink.[1,2] Currently, the only disposal methods available to producers in Minnesota without special approval from the Board of Animal Health are burial, incineration, rendering, and composting. Each of these methods has significant drawbacks.

Alternative methods of dead pig disposal should be biologically sustainable, environmentally safe, and cost effective, and should maximize the biosecurity of swine herds. In addition, any carcass disposal method must be acceptable to producers and communities. There should be minimal health risks for the public and adjoining livestock herds and flocks. In addition, the disposal method should have little or no impact on surface or ground water quality and should not cause odor problems. Recent research results suggest that depositing ground pig carcasses into an existing manure handling system may be an acceptable method of dead pig disposal.[3] With this method of animal disposal, nutrients in the carcass would be contained and judiciously applied to cropland with the nutrients present in swine manure. However, there is concern that this method may potentially contaminate soil and water with swine pathogens that may be present in the carcass at the time the manure is applied to the land. For example, Ellis and McCalla[4] reported that *Salmonella* can survive in soil from 7–168 days. Several factors, such as moisture, temperature, sunlight, pH, competitive organisms, organic matter, and soil type influence survival of bacteria in soil. Generally, presence of most pathogens in liquid manure is reduced as the duration of the storage period increases.[5] *Salmonella anatum* survived between 4 and 14 days and pseudorabies virus (PRV) survived ≤12 hours in liquid swine manure containing ground pig carcass stored at 25°C (77°F).[6]

The relatively short survival time of bacterial and viral pathogens when dead pigs are disposed of in the manure handling system limits the potential for pathogens to contaminate the environment, as long as producers observe a short waiting period between the time pig carcasses are added to the manure and when they apply that manure to the soil. However, producers may fail to observe the appropriate waiting period, which would create a potential risk of disease transmission. Therefore, the first objective of this experiment was to determine the survival of a bacterial (*S. anatum*) and viral (coliphage) organism in soil under cropping conditions that received liquid swine manure containing ground pig carcasses. A second objective was to determine the effects of manure application method on survival of the selected bacterial and viral organisms.

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The authors gratefully acknowledge the Minnesota Pork Producers Association for the financial support of this project, and the technical help of Doug Williams

Materials and methods

Preparing carcass/manure mixture

This experiment was conducted at the University of Minnesota West Central Experiment Station in Morris, Minnesota. Piglets (less than 5.5 kg [12 lb]) that died from various causes were collected from the swine research herd and frozen until they could be processed. Piglets were homogenized using a commercial sized grinder (Bioreducer™, Bio Quest Inc., Laurel, Nebraska) which is similar to a garbage disposer. Homogenized pig carcasses (31 kg [68 lb] @ 8% dry matter) were placed in two large buckets (capacity = 18.9 L [5 gal] per bucket). *Salmonella anatum* (total = 6×10^{11} CFU) and T_1 coliphage (total = 3.1×10^{12} PFU) were added in two equal portions to each bucket. The T_1 coliphage was used as a model to mimic the survival of human and animal viruses.[7] Bacteria and virus were mixed thoroughly with homogenized pig carcasses using a large stirring rod. The pig carcass/pathogen mixture was added to the reservoir tank of the manure applicator. Liquid swine manure collected from an anaerobic pit under a swine finishing building and pig carcass homogenate were mixed thoroughly by a recirculating pump mounted on the reservoir tank of the manure applicator. Liquid swine manure contained 5.66% dry matter.

The United States Department of Agriculture (USDA) Soil Conservation Service[8] estimated that manure production on a dry matter basis is:

- 1.1 kg (2.5 lb) for gestating sows,
- 2.7 kg (6.0 lb) for lactating sows, and
- 4.8 kg (10.6 lb) for nursery pigs

per 454 kg (1000 lb) of liveweight per day.

From these estimates, a breeding sow and her progeny would generate 359 kg (790 lb) of manure dry matter annually in a farrow-to-feeder pig operation. We estimated that each litter would generate two stillborn pigs (1.4 kg [3 lb] per pig) and two liveborn pigs that died before weaning (2.3 kg [5 lb] per pig). These dead pigs from two litters per year would contribute 2.9 kg (6.4 lb) of dry matter to the waste generated by a sow annually. The dead pig carcasses would represent about 0.8% ([2.9 kg/359 kg] × 100) (or [(6.4 lb/790 lb) × 100]) of the total waste dry matter generated by the sow. Note that only swine mortalities during the farrowing and lactation phases were considered. The disposal method described herein seems to have the greatest potential for disposing of pigs that die before or shortly after weaning. To create a "worst case" scenario, carcass dry matter was added to the liquid manure at 5% of the dry matter in the liquid manure. This concentration was calculated to be greater than five times the amount of carcass dry matter that would be generated from farrowing house mortalities in a commercial swine farrowing operation.

Applying carcass/manure mixture to soil

The following treatments were applied to experimental plots of cropland:

- liquid swine manure applied to the surface of cropland with no immediate tillage (SUR);
- liquid swine manure subsurface injected into soil (INJ);
- liquid swine manure containing homogenized piglet carcasses applied to the surface of cropland with no immediate tillage (SUR-P);
- same as SUR-P but manure was subsurface injected (INJ-P); and
- nonmanured negative control plot (CON).

Experimental plots received manure at a rate of 33,600 L per hectare (3600 gal per acre), which supplied an estimated 145 kg N per hectare (130 lb N per acre) to target a yield goal for corn of 8000 kg per hectare (130 bushels per acre). SUR and INJ treatments were applied to the appropriate plots first to prevent contaminating the manure applicator and cross-contaminating the experimental plots with *S. anatum* and coliphage (Figure 1-B).

Experimental plots measured 3 m × 10 m (10 ft × 35 ft) and were located on Nutley clay (Udertic Haploboroll moderately well-drained) soil. This soil has the following characteristics on an air-dry basis:

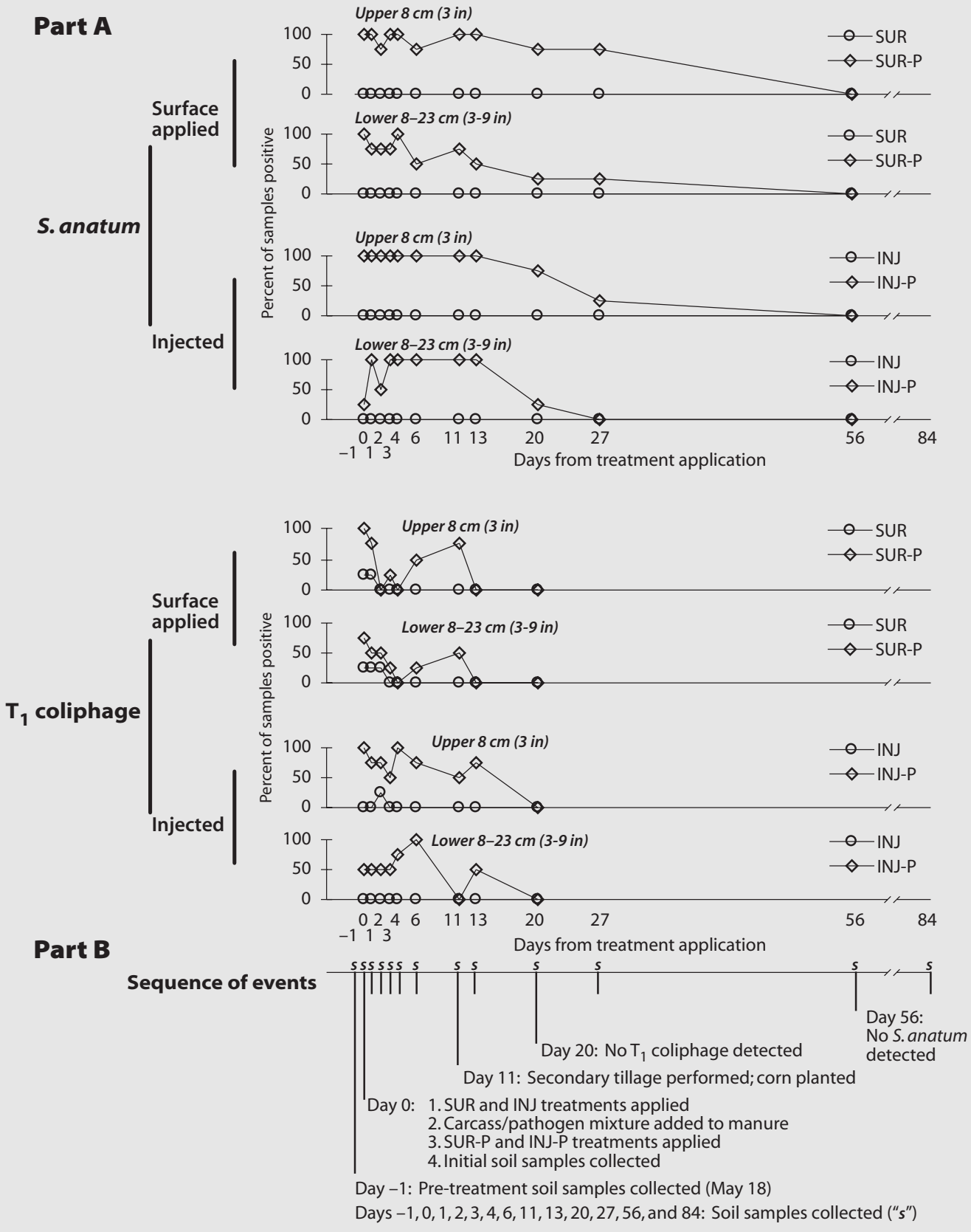
- 8 ppm nitrate nitrogen (0–24 inch depth),
- 17 ppm available phosphorus,
- 213 ppm exchangeable potassium,
- pH = 7.5, and
- 5.5% organic matter.

Primary tillage was accomplished with a chisel plow in the fall of 1994 following an oat crop. Initial spring tillage was performed using a field cultivator equipped with 15 cm (6 inch) sweeps. A plot-size manure applicator with helical screw pumps was used to provide accurate manure application rates. The applicator had four outlet ports mounted on 75-cm (30-inch) centers. Manure was broadcast on the surface of the plot for the SUR and SUR-P treatments. Manure for the INJ and INJ-P treatments was injected at a depth of about 10 cm (4 inches) using horizontal sweep injectors that were 40 cm (16 inches) wide. The seedbed was prepared using conventional secondary tillage and planting was delayed 11 days after the manure was applied. Corn (Pioneer variety 3095) was planted in plots at 74,000 seeds per hectare (30,000 seeds per acre). Weeds were controlled using a pre-emergence application of Lasso™ (alachlor) (Monsanto Company, St. Louis, Missouri) at 3.36 kg active ingredient per hectare (3 lb per acre) plus Bladex™ (cyanazine) (American Cyanamid Company, Wayne, New Jersery) at 2.47 kg of active ingredient per hectare (2.2 lb per acre) followed by row cultivation when corn plants were at the five-leaf stage. Herbicide was applied on May 31, 1995 and cultivation was performed on June 27, 1995. Corn was harvested at maturity with a plot combine. Yield and moisture content of grain were determined for each plot. Grain yields were adjusted to 15.5% moisture.

Soil sampling and analysis

Four soil samples were collected with a hand soil probe at the 0–8 cm (0–3 inch) and 8–23 cm (3–9 inch) depths from each experimental plot. Soil samples were collected the day before manure was applied, within 6 hours after manure was applied, and on days 1, 2, 3, 4, 6, 11, 13, 20, 27, 56, and 84 after manure was applied. Soil samples were collected between 14:00 and 18:00 hours on the day of sampling. Samples were refrigerated at 4°C overnight in sealed plastic bags and

Figure 1



Percent of samples containing detectable *S. anatum* and T_1 coliphage in the upper 8 cm (3 in) and lower 8–23 cm (3–9 in) of the soil profile (4 samples per treatment per sampling day) and sequence of events throughout the soil sampling period of the experiment.

transported in an ice-packed chest to the Veterinary Diagnostic Laboratory in St. Paul, Minnesota. Less than 24 hours elapsed between the time the soil samples were collected and the time they were processed for isolation of bacteria and phage.

The presence of *S. anatum* was detected in soil samples using standard methods.[9] Briefly, 2 g of soil sample (as-is basis) were added to Rappaport-Vassiliadis broth. After incubation at 37°C for 24 hours, the broth was plated on brilliant green agar plates. After incubation for another 24 hours, suspect colonies were subjected to biochemical identification using triple sugar iron agar, dextrose, lysine iron agar, sulfur indole motility agar, and urea tests.

Phage was isolated using the procedures of Gerba[7] by suspending 2 g of soil sample in 18 mL of phosphate-buffered saline. The suspension was allowed to settle for 10 minutes and 5 mL of supernatant from the top of the tube was filter sterilized. Serial tenfold dilutions of the filtrate (from 10^{-1} to 10^{-5}) were prepared in Luria-Berteni (LB) broth and mixed with 6- to 24-hour culture of host *Escherichia coli*. This mixture was then added to molten top agar, mixed, and spread on LB bottom agar plates. The plates were screened for phage plaques after incubation for 24 hours at 37°C.

Statistical analysis

A logistic regression procedure using maximum likelihood estimation of model parameters[10] was used to analyze categorical data (presence or absence) for survival of *S. anatum* and coliphage. The initial model considered the effects of treatment, block, day, and depth of sampling. Least-squares analysis of variance using General Linear Models[10] was used to analyze the effects of treatment and block on yield and moisture content of corn grain harvested at the end of the experiment. Mean separation for yield and moisture data was achieved using nonorthogonal designed contrasts.

Results and discussion

Manure was applied to experimental plots on May 19, 1995. The last soil sample was collected on August 10, 1995. During this experimental period, the average daily high and low air temperature was 28°C and 13°C (82°F and 55°F), respectively. Rainfall total was 25.7 cm (10.1 inches) and 1454 corn-growing degree-days ([daily maximum temperature + daily minimum temperature] ÷ 2 – 50°F) were recorded. The 100-year average rainfall was 23.4 cm (9.22 inches) and there were 1412 corn-growing degree-days. Corn was harvested on October 12, 1995. Neither corn yield nor moisture content of grain were different ($P > .25$) for SUR-P and INJ-P treatments compared to SUR and INJ treatments (Table 1).

None of the soil samples collected from plots the day before manure was applied contained detectable *S. anatum* or coliphage. The nonmanured negative control plot (CON) contained no detectable *S. anatum* or coliphage initially or at any time throughout the experiment. The absence of sentinel organisms in the CON plots indicates no cross-contamination of plots as a result of the methods used to sample soil.

Table 1

Influence of swine manure containing ground piglet carcass on yield and moisture content of corn grain

Treatment[a]	Grain yield	Grain moisture
CON	101.3 bu/acre	23.9 %
SUR	139.7	23.6
INJ	151.2	23.1
SUR-P	143.3	23.4
INJ-P	156.4	23.0
Pooled SE	*3.69* [b,c]	*0.25*

a CON = nonmanured negative control; SUR = liquid swine manure applied to surface of cropland; INJ = liquid swine manure subsurface injected into soil; SUR-P = liquid swine manure containing homogenized piglet carcasses applied to surface of cropland; INJ-P = liquid swine manure containing homogenized piglet carcasses subsurface injected into soil

b CON vs. all others ($P<.01$)

c SUR + SUR-P vs INJ + INJ=P ($P<.05$)

Salmonella anatum

No *S. anatum* was detected in the SUR and INJ treatments throughout the experimental period (Figure 1-A). *Salmonella anatum* was detected in a high proportion of SUR-P and INJ-P plots initially. This proportion declined over the course of the experiment ($P < .01$). By day 56 of the experiment, *S. anatum* could not be detected in any soil samples and was confirmed negative by soil samples collected on day 84. This period of survival for *Salmonella* is well within the range of 7–168 days reported by Ellis and McCalla.[4]

Treatment × day ($P < .01$) and treatment × depth × day ($P < .05$) interactions were observed when all four manure treatments were included in the statistical model (Table 2). These interactions were expected because no *S. anatum* was applied with the SUR and INJ treatments. A second analysis that included only the SUR-P and INJ-P treatments revealed no difference in survival of *S. anatum* due to method of manure application (surface applied versus injected). However, a main effect of depth ($P < .001$) was noted with no significant interactions between treatment and depth. Consequently, a third analysis that included SUR-P or INJ-P and depth of sample was conducted to determine the effect of sample depth on survival of *S. anatum*. A longer survival time for *S. anatum* was observed in the upper 8 cm (3 inches) of the soil profile compared with the lower 8–23 cm (3–9 inches) (Figure 1-A).

Salmonella anatum survived at least 27 days in some of the experimental plots. Because of this survival time, one may theorize that *Salmonella* could contaminate corn growing in these plots as the plant pushed its way through the soil. Ayanwale, et al.,[11] fertilized land with human sewage sludge that contained *Salmonella newport* C2. Corn silage harvested from this land contained no detectable *Salmonella* after ensiling and goats fed the corn silage for 17 months remained negative

for *Salmonella* infections. Furthermore, vegetables growing on the same land that produced the corn for ensiling were negative for *S. newport* C2. The potential for transfer of *Salmonella* from soil to harvested livestock feed seems small. However, potential transfer of *Salmonella* from soil to grazing animals has not been studied to our knowledge.

Coliphage

Soil from a small portion of plots that received the SUR and INJ treatments yielded coliphage on days 0, 1, and 2 (Figure 1-A). This observation is confusing because these plots had no coliphage on the day before application of manure and there was no coliphage-contaminated pig carcass added to the manure for these treatments. Furthermore, analysis of the manure before ground pig carcasses were added did not reveal the presence of coliphage. Aerosol contamination of plots assigned to the SUR and INJ treatments may have occurred when the SUR-P treatment was applied to adjacent plots. However, this cross contamination of SUR and INJ plots seems unlikely because these plots remained negative for *S. anatum* throughout the experiment and no coliphage was detected in CON plots. The assay procedure for coliphage is less sensitive than the assay for *S. anatum* because of the requirement to elute the virus from soil before culturing. Inefficient extraction of virus is inherent in the elution process. These insensitivities were further evident by the variable presence of coliphage in the contaminated plots throughout the experiment. However, all of the SUR and INJ plots yielded no coliphage by day 3 of the experiment and remained negative throughout the study.

Table 2

Results of logistic regression procedures using maximum likelihood estimation of model parameters

A. Treatments included in model: SUR, INJ, SUR-P, INJ-P

	Significance level	
Model parameter	***Salmonella*** (n = 352)	**Coliphage** (n = 288)
Day	.01	.001
Treatment	.001	.001
Depth of sample	.05	.13
Day × treatment	.005	.18
Day × depth of sample	.07	.84
Treatment × depth of sample	.005	.20
Day × treatment × depth of sample	.05	.70

B. Treatments included in model: SUR-P, INJ-P

	Significance level	
Model parameter	***Salmonella*** (n = 176)	**Coliphage** (n = 144)
Day	.001	.001
Treatment	.65	.01
Depth of sample	.001	.10
Day × treatment	.29	.89
Day × depth of sample	.45	.98
Treatment × depth of sample	.90	.35
Day × treatment × depth of sample	.14	.99

C. Treatment included in model: SUR-P

	Significance level	
Model parameter	***Salmonella*** (n = 88)	**Coliphage** (n = 72)
Day	.001	.01
Depth of sample	.01	.58
Day × depth of sample	.81	.77

D. Treatment included in model: INJ-P

	Significance level	
Model parameter	***Salmonella*** (n = 88)	**Coliphage** (n = 72)
Day	.001	.005
Depth of sample	.05	.10
Day × depth of sample	.15	.64

CON = nonmanured negative control;
SUR = liquid swine manure applied to surface of cropland;
INJ = liquid swine manure subsurface injected into soil;
SUR-P = liquid swine manure containing homogenized piglet carcasses applied to surface of cropland;
INJ-P = liquid swine manure containing homogenized piglet carcasses subsurface injected into soil

The plots assigned to the SUR-P and INJ-P treatments were negative for coliphage by day 20 of the experiment (Figure 1-A) and this was confirmed negative by samples collected on day 27. Statistical analysis of data which included all four manure treatments revealed important effects of treatment (P <.001). Effects could be influenced largely by the SUR and INJ treatments that yielded no coliphage for much of the experiment. A second analysis including effects of SUR-P, INJ-P, depth of sampling and day of sampling showed main effects of treatment (P <.01) and day (P <.001) with no significant interactions (Table 2). Survival of coliphage seemed to be greater in plots assigned to the INJ-P treatment compared with similar plots in the SUR-P treatment (Figure 1-A). Injecting coliphage under the surface of the soil may have protected the virus from ultraviolet radiation and warm temperatures caused by sunlight. Within the SUR-P treatment there was no significant effect of sampling depth on survival of coliphage (Figure 1-A). However, survival of coliphage in the INJ-P plots tended to be greater (P <.10) in the upper 8 cm (3 inches) compared with the lower 8–23 cm (3–9 inches) of soil. This higher survival rate may be explained by the increased concentration of bacteria in the top 8 cm (3 inches) of soil. Alexander[12] reported four to five times more bacteria in the top 2–8 cm (1–3 inches) of soil compared with soil at the 20- to 25-cm (8- to 10-inch) depth. Since coliphage attacks *E. coli*, the survival of coliphage may have been increased because of a larger population of hosts.

Viruses do not grow outside a living host and their survival is limited. Previous research conducted in our laboratory demonstrated that PRV and porcine reproductive and respiratory syndrome virus survived no more than 12 hours in swine slurry that contained ground piglet carcasses.[13] Garcia-Sirera, et al.,[14] composted PRV-contaminated pig carcasses and reported the virus did not survive 35 days of composting. These results, along with the findings of the experiment reported herein, suggest that survival of viruses in environments outside the host organism is relatively short.

Adding ground pig carcasses to manure management structures does increase the quantity of nutrients in the manure management system but this increase is insignificant. We estimate that pig carcass dry matter added to the manure produced by a breeding sow at 1% of the total dry matter generated would add about 0.6% nitrogen, 1.2% phosphorus, and 0.25% potassium to the manure.

This carcass disposal provides a convenient option for producers to dispose farrowing house mortalities in an environmentally friendly way. This is especially helpful when other methods of disposal either are not available or are not practical. However, swine veterinarians should be cautious about recommending use of this disposal method if waste water from manure management structures is being recycled into rooms that house pigs. Recycling wastewater that contains swine pathogens from ground pig carcasses may increase the potential for spread of disease to the rest of the swine herd.

Implications

- Under the conditions of this experiment, *S. anatum* and T_1 coliphage survived ≤56 and ≤20 days in soil, respectively.
- Method of manure application (surface versus injected) did not seem to influence survival of *S. anatum* significantly.
- Surface applied manure reduced survival of coliphage about 7 days compared with subsurface injection of manure.
- The potential for long term contamination of soil used for row crop production with *S. anatum* and pathogenic viruses when manure containing ground pig carcasses is applied to the soil seems remote.

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