

## Fate of *Salmonella* following application of swine manure to tile-drained clay loam soil

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### ABSTRACT

Land application of animal manure is an important means of utilization of nitrogen and phosphorus. However, the presence of pathogens in manure and their occasional leaching into subsurface water has become a topic of concern during the past few years. This study was undertaken to determine the extent to which *Salmonella anatum* may leach through tile-drained clay loam soil on which swine manure has been applied. For this purpose, swine manure was experimentally contaminated with *S. anatum* and applied to three tile-drained plots in winter of 2001 while another three plots served as negative controls. Following rainfall events in the spring of 2002 the tiles started to flow and leachate samples of subsurface water were collected at various time intervals and tested for *S. anatum*. *Salmonella anatum* was not found to leach into the subsurface drainage water indicating that it was either retained in the upper layers of soil or did not survive over winter. The leaching of faecal coliforms and coliphages was also tested. Faecal coliforms and coliphages were detected in the subsurface water from both manure and control plots, indicating the ability of the tile drainage system to transport these organisms to groundwater as the water percolates through the soil. Additional temporal studies over a longer time period are needed to determine the survival and leaching of pathogens and indicators into subsurface water.

**Key words** | clay loam soil, faecal coliforms, F<sup>+</sup>RNA coliphages, *Salmonella anatum*, somatic coliphages, swine manure, tile drainage

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### INTRODUCTION

Faecal contamination of groundwater from land application of human or animal wastes is one of the causes of deteriorating water quality that may have a direct public health impact. Since groundwater is an important source of drinking water in the United States and is often used without treatment, it has been a vehicle of many water-borne disease outbreaks (Moore *et al.* 1992). Although the use of animal manure on land provides an opportunity for recycling of nitrogen and phosphorus that is beneficial for crops (Gagliardi & Karns 2000), the animal manure may contain bacterial, viral, or protozoan pathogens of animal and human concern. The pathogen level in manure depends on the source animal, the animal's state of health and how the manure was stored and treated before use. If present in sufficient quantities, the pathogens may

contaminate surface and subsurface waters through runoff and leaching (Gagliardi & Karns 2000) creating potential problems for human and animal health.

During the last two decades, most environmental concerns about animal manure management have focused either on the effects of nutrients (especially nitrogen and phosphorus) on water quality, or have emphasized odours and air quality problems. Microbes present in manure are often considered to be of low priority despite the fact that several outbreaks of gastroenteritis have been traced to livestock operations (Jack & Hepper 1969; Pell 1997). Infections that may result from inadequately treated animal slurry include salmonellosis, tuberculosis, paratuberculosis, leptospirosis, anthrax, and clostridial infections (Jones 1980). *Salmonella*, an important pathogen of

humans and animals, has been isolated from pig slurry (Jones & Hall 1975) and has been reported to survive for more than 30 months in manure in experimental studies (Gibson 1965; Morse 1974).

Recently, application of swine manure on tile-drained soil has become a focal point of controversy throughout the swine producing areas of the United States and is becoming a matter of concern for pork producers (Keenlside 1998). It is believed that pathogens from manure may leach into the subsurface water through tile drainage (Thornley & Bos 1985; Dean & Foran 1992) but little information is available on the enrichment of subsurface tile drainage water with pathogens following land application of swine manure.

In addition to bacterial pathogens, a number of animal viruses may also be present in swine manure e.g. foot and mouth disease virus, swine fever virus, pseudorabies virus, transmissible gastroenteritis virus, porcine enteroviruses, porcine adenoviruses, and porcine reproductive and respiratory syndrome virus (Derbyshire & Brown 1978; Goyal 1993; Haas *et al.* 1995). Traditionally, faecal coliforms have been used as indicators of faecal contamination of water (*Standard Methods* 1998). However, these bacteria have been found to be inadequate in predicting the virological quality of water partly because viruses are more resistant to environmental conditions (including disinfection) than bacteria (Feachem *et al.* 1983; Richards 1985; Kator & Rhodes 1994). Both male-specific (F<sup>+</sup> RNA) and somatic coliphages have been proposed as alternative indicators for determining the presence of human and animal viruses in water (De Bartolomeis 1988; Kator & Rhodes 1994). This study was undertaken to compare the survival and leaching of *Salmonella*, faecal coliforms and male-specific and somatic coliphages following land application of swine manure on tile-drained clay loam soil.

## METHODS

### Swine manure

Ten-week-old liquid swine manure was obtained from an anaerobic pit under a swine-finishing barn. The manure

was experimentally contaminated with *S. anatum* at  $2 \times 10^6$  cfu/litre ( $9 \times 10^6$  cfu/gallon) of manure and thoroughly agitated prior to and during application of manure on field plots. The bacterial culture was prepared in gram-negative broth following the method of Ajariyakhajorn *et al.* (1997). The manure was injected in the soil using a manure applicator during November 2001. Since faecal coliforms and coliphages are normally present in swine manure, it was not considered necessary to artificially contaminate swine manure with these agents. Manure was not tested for pathogens before application on land.

### Tile drainage plot set-up

Six tile-drained clay loam soil plots, routinely used for corn cultivation, are located at the Southern Research and Outreach Center at Waseca, MN. These plots (15 m × 16.7 m) were demarcated and separated by 12-mm plastic sheeting trenched to a depth of 2 m to minimize lateral flow from one plot to another. A 10-cm perforated plastic tile installed at a depth of 1.33 m and located 1.67 m from one end of the plot was used to drain each plot separately. Culverts installed vertically to a depth of 2 m allowed sample collection from each plot. Manure contaminated with *S. anatum* was applied to three plots at the rate of 9000 litres per hectare (5000 gal/acre). Another three plots were maintained as negative controls without application of swine manure. However, these negative control plots were treated with urea (29 kg N/hectare (160 lb N/acre)) in April 2002, followed by corn plantation.

### Sampling schedule and experimental design

Subsurface drainage into the tile lines began following rainfall on 3 June 2002 (date of first sampling) and ended on 8 July 2002 (last sampling date). Using aseptic techniques, leachates were collected weekly for 6 weeks from each individual plot. The samples were maintained at 4°C and usually tested within 24 h of collection for *S. anatum*, faecal coliforms and coliphages (F<sup>+</sup> RNA and somatic coliphages).

### ***Salmonella anatum***

The method recommended in section 9260 B of *Standard Methods for the Examination of Water and Wastewater* (1998) was followed. Leachate samples (100 ml volume) were filtered through 0.45  $\mu\text{m}$  membrane filters. The membranes were rolled and placed in tubes of tetrathionate broth (Becton Dickinson, Cockeysville, MD) for enrichment. After incubation at 37°C for 24 h, a loopful of the culture was streaked on brilliant green agar (Becton Dickinson, Cockeysville, MD). The number of suspect colonies was counted (cfu/100ml) after incubation at 37°C for 24 hr and a representative number was picked and inoculated in triple sugar iron agar and urea agar (Becton Dickinson, Cockeysville, MD) for confirmation.

### **Faecal coliforms**

The membrane filter technique as recommended in section 9222 D of *Standard Methods for the Examination of Water and Wastewater* (1998) was followed. Briefly, 100 ml volumes of water samples were filtered through 0.45  $\mu\text{m}$  membrane filters. These filters were then placed on mFC agar plates (Becton Dickinson) followed by incubation at 45°C for 24 h. The results were reported as cfu/100 ml.

### **F<sup>+</sup>RNA (male-specific) coliphages**

The single agar layer (Environmental Protection Agency 2001) procedure was used to detect male-specific coliphages. A log-phase culture of the host bacterium (*E. coli* Famp, ATCC no. 700891) was prepared by inoculating 1 ml of an overnight culture in 100 ml of tryptic soy broth (Becton Dickinson) followed by incubation for 4 hr at 37°C on a shaker platform at 100 rpm. To 100 ml of a sample of subsurface water were added 0.5 ml of 4 M  $\text{MgCl}_2$ , 10 ml of log-phase culture of *E. coli* F<sub>amp</sub>, and 100 ml of molten double strength tryptic soy agar. Each sample was thoroughly mixed and poured into four 150 × 15 mm Petri dishes followed by incubation at 37°C for 24 h. Positive results were indicated by circular zones of lysis in contrast to opaque lawns of host bacterial

growth. Plaques from all four plates were counted for each sample and expressed as pfu/100 ml.

### **Somatic coliphages**

The procedure used for somatic coliphages was the same as used for F<sup>+</sup> RNA phages except that *E. coli* CN13 (nalidixic acid resistant mutant of *E. coli*; ATCC 700609) was used as the host bacterium.

## **RESULTS AND DISCUSSION**

The occurrence of pathogens in subsurface water is shown in Table 1. *Salmonella anatum* was not detected in any of the 36 water samples indicating that this organism either does not survive over winter or is retained by the upper layers of soil and is not transported to groundwater. Degradation of microorganisms either by self-decomposition or elimination by soil microorganisms is also possible (Platz 1980).

Surprisingly, the numbers of all indicators (faecal coliforms, male-specific phages and somatic phages) were similar in both control and manure applied plots (Table 1). These numbers were high in week 1 and 2 samples but declined thereafter. This may represent background levels of these microorganisms whose source may have been faecal material deposited by animals, rodents and birds frequenting these plots. Increases in faecal coliform counts in the subsurface water have been reported following the grazing of cattle on agricultural fields (Howell *et al.* 1995). It was not possible to screen the tile drainage prior to application of swine manure because tile drainage starts only after rainfall. As the fields were not protected by fencing, the probability of faecal material deposition by grazing or wild animals cannot be ignored.

Male-specific coliphages were detected in all 36 leachate samples. The average numbers of male-specific phages were higher at week 1 in both urea (control) and manure plots, and thereafter the numbers declined and were similar in manure and urea applied plots during the study period. No significant correlation was observed

**Table 1** | Mean values of total faecal coliforms and coliphages detected in leachate samples

Organisms	Treatment	Mean (standard deviation)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
<i>Salmonella anatum</i> <sup>a</sup>		0.0	0.0	0.0	0.0	0.0	0.0
Faecal coliforms <sup>b</sup>	Urea	37.3 (62.9)	45.3 (71.7)	0.0	8.7 (10.9)	0.3 (0.6)	0.0
	Manure	3.7 (2.3)	34.3 (15.0)	0.0	8 (6.0)	0.3 (0.6)	0.0
FRNA coliphages <sup>c</sup>	Urea	43.3 (27.3)	12.3 (11.2)	2.0 (0.0)	14.0 (6.0)	1.0 (1.0)	7.7 (4.7)
	Manure	32.7 (14.5)	10.7 (12.7)	3.7 (3.5)	4.7 (3.8)	19.7 (18.0)	9.0 (6.6)
Somatic coliphages <sup>c</sup>	Urea	17.7 (12.6)	22.0 (8.1)	2.3 (2.5)	0.6 (0.6)	2.7 (2.5)	10.7 (6.0)
	Manure	6.3 (10.1)	27.3 (18.7)	22 (36.4)	4.7 (8.0)	3.3 (3.2)	8.3 (5.5)

<sup>a</sup>*Salmonella anatum* was not detected in any of the 36 samples.

<sup>b</sup>Faecal coliforms were expressed as cfu/100 ml.

<sup>c</sup>Coliphages were expressed as pfu/100 ml.

between control and manure treated plots for male-specific coliphages. The numbers of somatic coliphages were lower in general than those of male-specific phages. In general, the total numbers of male-specific and somatic coliphages was relatively higher than those of bacterial indicators in all plots. The isolation of bacterial and viral indicators from subsurface drainage water indicates the ability of the tile drainage system to transport these organisms to groundwater as the water percolates through the soil. The occurrence of coliphages in the absence of faecal coliforms is in agreement with earlier studies in which enteric viruses were found in groundwater even in the absence of faecal coliforms (Zohar *et al.* 1984; Alhajjar *et al.* 1988).

Direct examination of water for human and animal enteric viruses is not practical because the methods for virus detection in water are time-consuming, expensive, and difficult to perform (Snowdon & Cliver 1989). In addition faecal coliforms, which are good indicators of bacterial pollution, are not very reliable for predicting the virological quality of water (Gerba & Goyal 1982). Hence, alternative indicators such as male-specific and somatic coliphages have been advocated as valuable indicators of

enteric viruses (Kator & Rhodes 1994). Higher numbers of coliphages observed in water (as compared to faecal coliforms), especially during weeks 3, 5 and 6 indicate that phages may survive longer than faecal coliforms and/or may travel longer distances. Hence, coliphages would appear to be better indicators of viral pollution than faecal coliforms. Since the numbers of male-specific phages were often higher than those of somatic phages, the former may be an even better indicator of faecal pollution in general and viral contamination in particular.

## CONCLUSIONS

In conclusion, results of this 1-year study suggest that if swine manure is applied late in the fall before freeze-up, the risk of subsurface contamination due to *S. anatum* percolation during the following spring is minimal. The isolation of faecal coliforms and viral indicators (coliphages) from subsurface drainage water, however, indicates the ability of the tile drainage system to transport these organisms to groundwater as the water percolates

through the soil. However, to give a solid recommendation on leaching of pathogens following swine manure application in cultivated fields additional long-term studies are needed.

## ACKNOWLEDGEMENTS

This study was supported in part by a grant from The National Pork Board (NPB Project no. 02-095). The technical assistance of Jeff Vetsch and David Groh is gratefully acknowledged.

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