Microbial Transport in Surface Runoff from Manure-Amended Soils

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Abstract

Surface application of manure to agricultural soils can contribute pathogens to runoff water. This rainfall simulation study assessed *Salmonella* and *E. coli* in surface runoff from packed stainless-steel soil boxes (100 cm long x 20 cm wide x 5 cm deep) amended with dairy slurry, liquid swine manure, poultry litter, and composted poultry litter. Two rainfall-simulation events were conducted four days apart with manure-amended soil (surface applied at a rate of 150 kg N ha^{-1}) and rainfall was delivered at 7 cm h^{-1} for 40 minutes. Runoff water was collected from soil boxes and analyzed for the presence of *Salmonella* and *E. coli*. These bacteria were not detected initially in poultry litter or poultry compost, but *E. coli* was detected in runoff water from poultry compost-amended soil in a range of 0.40-1.15 log_{10} CFU/ml. However, initial concentrations of *Salmonella* and *E. coli* in dairy slurry were 4.60 and 6.61 log_{10} CFU/g, respectively, whereas initial concentrations of these bacteria in liquid swine manure were 4.08 and 5.08 log_{10} CFU/ml, respectively. *Salmonella* and *E. coli* were both detected in runoff water from dairy slurry- and liquid swine manure-amended soil during the first rainfall simulation event at concentrations of 2.0-3.2 log_{10} CFU/ml and 4.0-4.3 log_{10} CFU/ml, respectively. *Salmonella* was not detected in runoff water from the second rainfall simulation, but *E. coli* was detected at concentrations of approximately 2.6 log_{10} CFU/ml from the manure-amended soils. Results showed that manure type and initial concentrations of pathogens affected pathogen concentrations in surface runoff water. In addition, pathogen concentrations were greater in runoff water immediately after manure application in comparison to concentrations four days later. This indicates that soil retention or die-off could have contributed to pathogen reduction in the interim.

Introduction

Microbial contamination of fresh produce from water can occur through various pathways, including irrigation water, natural or controlled flooding, agricultural sprays, or runoff. Animals, both wild and domestic, are known reservoirs of pathogenic microorganisms such as *E. coli* O157:H7 and *Salmonella* that can be shed into the environment through animal feces. Manure, whether fresh or sometimes aged, continues to be regularly land applied as a fertilizer for crop production in both organic and conventional farming operations. When manure is not subjected to any type of storage phase or treatment practice, such as composting or ageing, pathogen populations in the manure can be relatively large. As a result, pathogens may be introduced into the food chain when manure is incorporated into the soil, introducing the potential for bacterial pathogens to move within the crop-soil environment. Water transport of pathogenic microorganisms from application sites in crop fields to off-site locations is an unintended consequence of manure use as fertilizer, even when applications are consistent with nutrient management recommendations. Widespread concerns remain about the potential for enteric bacteria and pathogens in manure to contaminate waterways through translocation by runoff.

Main Objective

The purpose of this study was to assess the microbial transport through runoff water from soils amended with various types of manure by obtaining quantitative data on *Salmonella* and *E. coli* that would translocate from the soil through water during a rainfall simulation events.

Materials and Methods

Soil and Manure Sources

Soil was obtained from the UMES research farm, air dried, and mixed thoroughly to a homogenous condition. Four manures types were selected to represent major livestock sources and different storage and handling processes. Liquid swine manure was collected from storage lagoons located on the University of Maryland Eastern Shore (UMES) swine facility. Dairy manure (slurry) was obtained from the Pennsylvania State College Farm, while broiler chicken litter and broiler chicken-litter compost were obtained from the UMES farm. Liquid swine manure and dairy manure, considered as fresh manures, had not been subjected to any extended storage period or manure treatment on-farm, but had been stored at 4°C for 2 weeks prior to application. Broiler chicken litter collected from the UMES farm was also considered to be fresh manure and was collected from an excess waste manure pile that was cleaned out from chicken houses, stacked, and left undisturbed for approximately 6-12 months before use in this experiment. The chicken-litter compost had been subjected to a 3-month composting process and subsequent storage of 11 months prior to its inclusion in this study.

Rainfall Simulation Experiments

Air-dried soil (12.2 kg) was placed into 1 m long x 20 cm wide x 5 cm deep stainless steel box with a back wall 2.5 cm higher than the soil surface and a 5 mm drainage hole in the base of each box. Four manure treatments were individually broadcasted onto the soil surface in the boxes at a rate of 150 kg N ha^{-1}. There was also one control treatment in which no manure was applied. Each treatment consisted of 4 replications. Two separate rainfall simulations were conducted 1 and 5 days after manure application with a portable rainfall simulator equipped with a TeeJetTM ½ HH SS 30 WSQ nozzle. Packed soil boxes were placed at a 3% slope during each rainfall simulation event with the rainfall nozzle positioned approximately 305 cm above the soil surface of each group of packed boxes. Rainfall lasted for 40 minutes and was delivered at approximately 7 cm h^{-1}. During the simulation, runoff was drained into glass jars that had been rinsed with acid and deionized water. Runoff water was collected from each box; and after thorough mixing, 50 milliliter subsamples of runoff water were individually extracted from the glass jars and held on ice until taken to the laboratory for microbial processing and analysis.

Figure 1: Rainfall simulation apparatus (A); and Collection of runoff water during rainfall (B)

Bacterial Sampling and Enumeration

Microbial analysis and enumeration was performed for *E. coli* (generic) and *Salmonella* on the runoff samples. Fresh manure treatment samples along with poultry compost and soil samples were analyzed initially to obtain baseline microbial counts in each respective treatment. Twenty-five grams of manure, compost, and soil samples (with the exception of liquid swine manure) were placed in Whirl-pak™ filter bags. Two hundred and twenty five milliliters of buffered peptone water (BPW) was added to each bag. Filter bags were stomached for 2 minutes at 200 rpm's. Ten milliliter subsamples were removed from the filter bags and placed into sterile 15 ml centrifuge tubes from which 50 µl samples were directly plated in duplicate onto selective media using an Autoplate® 4000 spiral-plater (Spiral Biotech, Bethesda, MD, USA). All plates were incubated at their respective temperatures and counted as described below.

Liquid swine manure and all runoff water samples were processed differently from the solid manure, compost, and soil samples. Aliquots (25 ml) of liquid samples were mixed with 25 ml of BPW in sterile 50 ml plastic tubes. After briefly vortexing, 10 ml subsamples were transferred to a sterile 15 ml plastic tube, which was then centrifuged at 250-500 rpms for 2 minutes to sediment large solids. The liquid suspension was transferred to a 15 ml sterile plastic tube and 50 µl was directly plated onto the selective media. Selective media used for enumeration included MacConkey agar with methylumbelliferyl glucuronide (MUG) supplement (MAC-MUG) for generic *E. coli* and Xylose Lactose Tergitol™ 4 (XLT_4) for *Salmonella*. All plates were incubated at optimal temperatures for microbial growth for 18-24 hours, with XLT_4 plates incubated at 37°C and MAC-MUG plates incubated at 44.5°C. After incubation, plates were observed and colonies counted using the segment pair counting method (Spiral Biotech, Bethesda, MD, USA) to obtain a final value for colony forming unit (CFU) ml^{-1}.

Data Analysis

Final concentrations of *E. coli* and *Salmonella*, expressed as CFU ml^{-1} and CFU g^{-1}, were log transformed to log_{10} CFU ml^{-1} and log_{10} CFU g^{-1}. Data were analyzed using Statistix v. 9 (Analytical Software, Tallahassee, FL, USA). Analysis of variance was used to compare the concentrations of *Salmonella* and *E. coli* transported in runoff water from each respective treatment in each rainfall simulation event as well as to compare the total concentrations of the organisms for each rainfall simulation event. The level of significance of $p<0.05$ was considered statistically significant. Tukey's HSD test was used to compare treatment means.

Results

Initial concentrations of *Salmonella* and *E. coli* in dairy manure were approximately 6.61 and 4.60 log_{10} CFU g^{-1} for *E. coli* and *Salmonella*, respectively, while initial concentrations of these organisms in liquid swine manure were approximately 5.08 and 4.08 log_{10} CFU ml^{-1} for *E. coli* and *Salmonella*, respectively (Fig. 2). These organisms were not detected initially in the soil control treatment, poultry compost, or fresh poultry litter. Although *E. coli* was not detected in the poultry compost initially, this bacterium was detected in runoff water of both rainfall simulation events at concentrations of approximately 1.15 and 0.40 log_{10} CFU ml^{-1} for rainfall simulation 1 and rainfall simulation 2, respectively (Fig. 3). *Escherichia coli* was detected in runoff water from dairy manure-amended soil during both rainfall simulation events at concentrations of 4.14 and 2.51 log_{10} CFU ml^{-1} for rainfall simulation 1 and rainfall simulation 2, respectively (Fig. 3). Likewise, the same organism was detected in runoff water from liquid swine manure-amended soil during both rainfall simulation events at concentrations of 4.34 and 2.53 log_{10} CFU ml^{-1} for rainfall simulation 1 and rainfall simulation 2, respectively (Fig. 3). *Salmonella* was detected in runoff water from dairy and liquid swine manure-amended soil in only the first rainfall simulation event (Fig. 3). The concentrations of this organism detected in water samples were approximately 2.03 and 3.03 log_{10} CFU ml^{-1} for water from dairy manure-amended soil and water from liquid swine manure-amended soil, respectively. Analysis of variance indicated that the manure treatments had a significant effect on the concentrations of *E. coli* ($p<0.01$) in both rainfall simulations and for *Salmonella* ($p<0.01$) in rainfall simulation 1

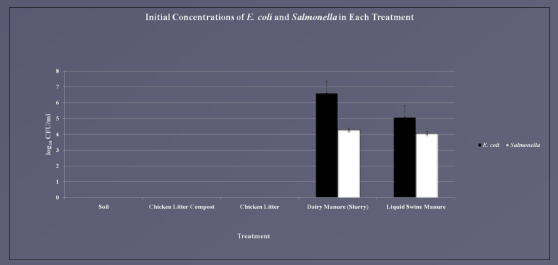

Figure 2: Initial concentrations (log_{10} CFU ml^{-1}/g^{-1}) of *Salmonella* and *E. coli* in soil and manure treatments. Error bars indicate standard error of the mean (n=4).

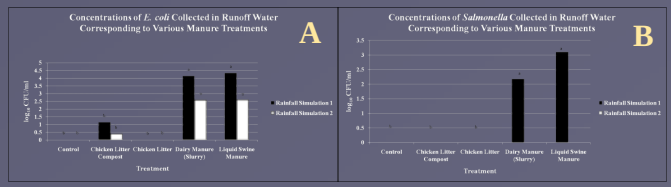

Figure 3: Concentrations (log_{10} CFU ml^{-1}) of *E. coli* in runoff water (both rainfall simulations) from packed soil boxes amended with various treatments (A); and Concentrations (log_{10} CFU ml^{-1}) of *Salmonella* in runoff water (both rainfall simulations) from packed soil boxes amended with various treatments (B); Means (n=4) for each respective rainfall simulation with similar letters are not significantly different at ($p<0.05$) according to Tukey's HSD All-Pairwise Comparisons Test

Conclusion

This study indicates that microorganisms present within manure can travel in the environment and subsequently serve as environmental infectious agents. Measures involving manure application and manure treatment should be taken to reduce the occurrence of pathogen spread by runoff water as much as possible. Our results substantiate the value of manure treatment in reducing the risk from *E. coli* and *Salmonella* in manure runoff and are consistent with previous reports from rainfall simulation studies with animal manure that show bacterial loading in initial runoff is greater than that from subsequent rainfall events.

Acknowledgements

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