Antibiotic Resistant and Pathogenic Bacteria Associated with Rain Runoff following Land Application of Poultry Litter

John P. Brooks
USDA-ARS, Waste Management and Forage Research Unit
810 HWY 12 E.
Mississippi State, MS 39762
662-320-7411
E-mail: jbrooks@msa-msstate.ars.usda.gov

ABSTRACT

Poultry rearing in the United States is approximately a thirty million dollar per year industry. To produce this large number of product, a by-product such as poultry litter is inevitable. The land application of poultry litter as an organic fertilizer is an ideal choice for the disposal of this high nitrogen, high organic waste. However, some precautions must be observed, particularly with regard to potential rain-mediated runoff of litter-related microorganisms following any large-scale precipitation events, such as those common to the Mississippi area. To investigate this phenomenon, poultry litter application will be simulated in a controlled greenhouse environment. Litter will be applied to Bermuda grass (*Cynodon dactylon*) plots held within runoff trays designed to simulate environmental conditions, by simulating slope, soil type, and climate. A rainfall simulator will be used to simulate precipitation events. Rainfall will be simulated on a weekly basis for a total of 4 consecutive weeks. Following each rain event, runoff and leachate samples will be collected for microbial analysis. Soil will be collected prior to the commencement of the experiment and following experiment termination. Total Heterotrophic Plate Count bacteria, antibiotic resistant bacteria, fecal coliforms, enterococci, staphylococci, *Clostridium perfringens*, and *Salmonella* spp. will all be investigated using both cultural and molecular methods. Select pathogens, indicator, and heterotrophic plate count bacteria will be selected for further genus typing and antibiotic resistance. In ad ition to the litter applied runoff trays, control trays in which no litter will be applied and chemical fertilizer applications will be used as comparison controls. Results from this experiment will lead to future in field application studies, under environmental conditions. These experiments will then be used to identify optimal field conditions necessary to achieve decreased potential for microbial runoff and subsequently provide much needed information to the area of litter application.

Keywords: Agriculture, Water Quality, Surface Water, Management and Planning

Introduction:

Land application of waste byproducts as fertilizer has been a common practice for man since the practice of land applying "night-soil" begun centuries ago. Wastewater treatment biosolids and manure now constitute the largest supplies of organic fertilizers. Manure from bovine, ovine, and poultry sources are some of the most common sources of organic fertilizers. These wastes are ripe with N, P, and other plant nutrients which make the application of these manures as fertilizer the most efficient method of manure reuse. However, despite the presence of these nutrients, manure land application can create some potential environmental hazards. Runoff of manure borne bacterial, viral, and parasitic pathogens has been demonstrated in a few studies (Entry et al., 2000; Stout et al., 2005; Thurston-Enriquez et al., 2005; Ferguson et al., 2007). Though many of these pathogens are environmentally labile organisms, some such as spore forming bacteria can survive for extremely long periods of time. Even fecal borne bacteria such as *Salmonella* and *Escherchia coli* can survive under relatively favorable environmental conditions (Jiang et al., 2002; Malik et al., 2004). In addition the presence of antibiotic resistance bacteria can compound the issue as potentially all manure borne bacteria (pathogenic and non-pathogenic) can harbor antibiotic resistance (both intrinsic and acquired). Horizontal transfer of mobile antibiotic resistance genes from one bacterium to another can potentially occur under environmental and in vivo conditions (Rensing et al., 2002; Dzidic and Bedekovic, 2003).

The purpose of this research was to identify the potential for manure borne bacteria to be horizontally transferred via surface water runoff following rain events. Runoff samples were collected from runoff plots and analyzed for the presence of a wide range of bacteria. Antibiotic resistance profiles were obtained from select isolates. Runoff samples were also collected from control plots without manure application.

Materials and Methods

Bermuda grass (*Cynodon dactylon*) was established in 3.1 sq ft troughs with non-sterile soil. Poultry litter (approximately 7 days old) was applied to the troughs at two organic fertilizer rates of 250 lb N, and 50 lb P per acre, a high and a low rate respectively. A third poultry litter treatment followed the 250 lb N treatment, however lime (CaO) was added at a 10% volume. Troughs with inorganic fertilizer (250 lb N, and 50 lb P per acre) were used as control fertilizer troughs. In addition, a trough with no fertilizer (organic or inorganic) applied was also used. Each trough was replicated in triplicate and placed onto carts in a randomized fashion. Troughs were held in a greenhouse from Dec. 06 through Feb. 07 and environmental conditions for optimal growth were met through the use of heat lamps and fans. Rain was generated via the use of a constructed rain simulator and was operated at 27 mm/hr. Rain events were as follows: 1, 3, 10, 17, and 25 days post application of litter, each lasting approximately 30 minutes. Each trough was rained upon equally and was raised to a height to simulate an approximate 3% slope.

Runoff water samples were collected from each trough following each rain event and were analyzed for microbial content including: Heterotrophic plate count bacteria (HPC), thermotolerant coliforms, total coliforms, *Staphylococcus*, *Enterococcus*, and *Clostridium perfringens* using modified standard methods. Antibiotic resistance profiles were generated from representative isolates. The Kirby Bauer method of assessing antibiotic resistance was used for antibiotic profiles of twelve antibiotics (Bauer et al., 1966).

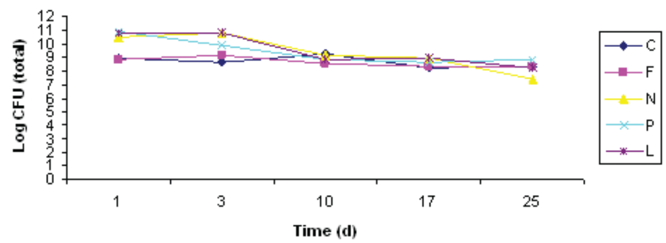

Figure 1. Heterotrophic plate count bacteria detected in runoff water samples. Values are presented as CFU present in the total runoff amount. C = Control troughs, F = Inorganic Fertilizer troughs, N = 250 lb/acre N poultry litter rate troughs, P = 50 lb/acre P poultry litter rate troughs, L = 250 lb/acre N poultry litter rate amended with 10% volume lime troughs.

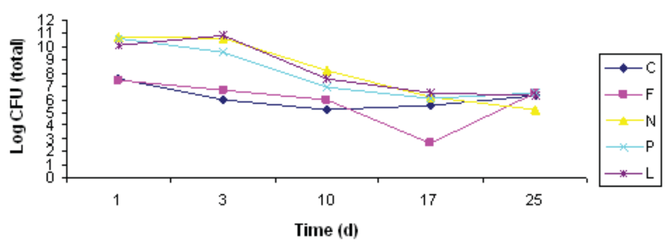

Figure 2. Staphylococcus detected in runoff water samples. Values are presented as CFU present in the total runoff amount. C = Control troughs, F = Inorganic Fertilizer troughs, N = 250 lb/acre N poultry litter rate troughs, P = 50 lb/acre P poultry litter rate troughs, L = 250 lb/acre N poultry litter rate amended with 10% volume lime troughs.

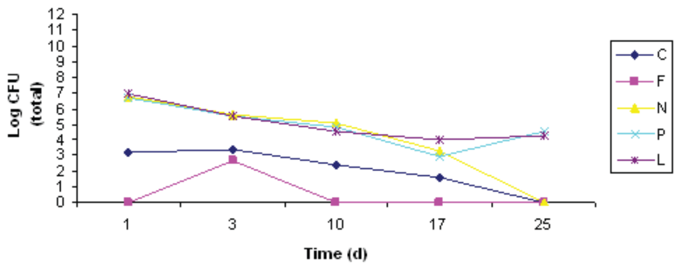

Figure 3. Enterococcus detected in runoff water samples. Values are presented as CFU present in the total runoff amount. C = Control troughs, F = Inorganic Fertilizer troughs, N = 250 lb/acre N poultry litter rate troughs, P = 50 lb/acre P poultry litter rate troughs, L = 250 lb/acre N poultry litter rate amended with 10% volume lime troughs.

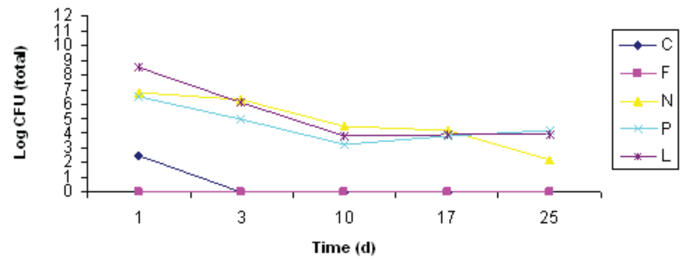

Figure 4. Clostridium perfringens detected in runoff water samples. Values are presented as CFU present in the total runoff amount. C = Control troughs, F = Inorganic Fertilizer troughs, N = 250 lb/acre N poultry litter rate troughs, P = 50 lb/acre P poultry litter rate troughs, L = 250 lb/acre N poultry litter rate amended with 10% volume lime troughs.

Results and Discussion

Overall an increase in the amount of bacteria detected in runoff was correlated with manure application (Figure1). Troughs with poultry litter application yielded relatively high total amounts of manure borne bacteria such as HPC, *Staphylococcus*, *Enterococcus*, and *C. perfringens* (Figures. 1, 2, 3, and 4 respectively). Both rates of manure application yielded these bacterial groups, with increasing totals associated with increasing manure rates. Chemical fertilizer, and no fertilizer controls yielded relatively less of these bacterial groups, up to 7 orders of magnitude less in the case of *C. perfringens*. These trends proceeded through the first 3 rain events and in the case of *C. perfringens* proceeded through the end of the rain events (25 days later). Although staphylococci and enterococci can both be found in the environment, the presence of enterococci can most likely be attributed to the presence of fecal contamination and is a fairly reliable indicator. Staphylococci on the other hand, can be readily present in non-impacted environments as many of these organisms are saprophytes. However, the overwhelming presence of these organisms associated with the land application of poultry manure lends credence to the idea that these could be used as potential indicators of poultry manure runoff. The majority of the bacterial groups associated with the poultry litter used in this study were staphylococci. Traditional fecal indicators such as coliforms and thermotolerant coliforms yielded no such trends as these were present in runoff from both organic and inorganic fertilizer applications (Figure 5). As such these organisms would not be able to be used as indicators of poultry manure horizontal movement. Since the soil used in the troughs was collected from the environment and not sterilized prior to use, this was a possibility, as many soils are known to have detectable levels of these organisms due to agricultural practices involving both organic and non-organic fertilizers. This presence renders them unable to be used as suitable tracers of manure movement.

Antibiotic resistance patterns may serve as suitable indicators of poultry manure application. However depend-

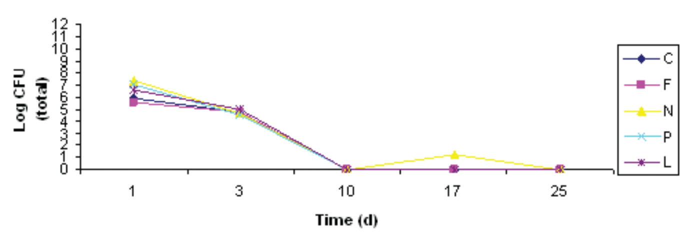

Figure 5. Thermotolerant coliform detected in runoff water samples. Values are presented as CFU present in the total runoff amount. C = Control troughs, F = Inorganic Fertilizer troughs, N = 250 lb/acre N poultry litter rate troughs, P = 50 lb/acre P poultry litter rate troughs, L = 250 lb/acre N poultry litter rate amended with 10% volume lime troughs.

ing on the source of the manure, the presence of traceable antibiotic resistance patterns may be difficult to use as some growers don't choose to use antibiotics in their feed regimens. Following the first and second rain events, antibiotic resistance patterns reflected more resistance (> 4 antibiotics) at a more consistent rate, consistent with the presence of manure. *Staphylococcus* isolates presented the most consistent antibiotic resistance patterns. Following the 3rd runoff event, antibiotic resistance patterns reflected that of the controls.

This research demonstrated that under favorable precipitation events (1 d post application) and conditions, poultry manure application can lead to microbial runoff. However the overwhelming presence of staphylococci, enterococci, and clostridia in poultry litter can lead to the exploitation of these organisms as indicators of poultry manure runoff. Antibiotic resistant bacteria were able to move with the runoff, however only a few isolates displayed antibiograms with resistance to greater than 4 antibiotics. This research will be repeated under field conditions using a similarly designed study to determine the effects of more realistic conditions.

References

Bauer, A.W., Kirby, W. M. M., Sherris, J.C., and Turck, M. 1966. Antibiotic susceptibility testing by standardized single disk method. American Journal of Clinical Pathology. 45: 493-496.

Dzidic, S., and Bedekovic, V. 2003. Horizontal gene transfer-emerging multidrug resistance in hospital bacteria. Acta Pharmacol. Sin. 24: 519-526.

Entry, J.A., Hubbard, R.K., Thies, J.E., and Fuhrmann, J.J. 2000. The influence of vegetation in riparian filterstrips on coliform bacteria: I. Movement and survival in water. Journal of Environmental Quality 29:1206-1214.

Ferguson, C.M., Davies, C.M., Kaucner, C., Krogh, M., Rodehutskors, J., Deere, D.A., and Ashbolt, N.J. 2007. Field scale quantification of microbial transport from bovine faeces under simulated rainfall events. Journal of Water and Health 5:83-95.

Jiang, X., Morgan, J., and Doyle, M.P. 2002 Fate of Escherichia coli O157:H7 in manure-amended soil. Applied and Environmental Microbiology 68:2605-2609.

Malik, Y.S., Randall, G.W, and Goyal, S.M. 2004. Fate of Salmonella following application of swine manure to tile-drained clay loam soil. Journal of Water and Health 2:97-101.

Rensing, C., Newby, D.T., and Pepper, I.L. 2002. The role of selective pressure and selfish DNA in horizontal gene transfer and soil microbial community adaptation. Soil Bio. Biochem. 34: 285-296.

Stout, W.L., Pachepsky, Y.A., Shelton, D.R., Sadeghi, A.M., Saporito, L.S., and Sharply, A.N. 2005. Runoff transport of faecal coliforms and phosporus released from manure in grass buffer conditions. Letters in Applied Microbiology 41:230-234.

Thurston-Enriquez, J.A., Gilley, J.E., and Eghball, B. 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. Journal of Water and Health 3.2:157-171.