Small farm plots and application of simulated rain to determine the potential for bacterial runoff after poultry litter surface application to bermudagrass

John Brooks and Ardeshir Adeli
USDA Agricultural Research Service, Waste Management and Forage Research Unit
john.brooks@ars.usda.gov

Land application of poultry litter is an economical and environmentally viable use of this manure by-product. However the recent concern associated with fresh produce and pathogenic bacterial contamination has led to increased scrutiny regarding land applied manures. Runoff following a rain event is one possible source of environmental contamination resulting from manure application. In this second part of a two-part study a series of treatments involving litter (two rates), inorganic fertilizer, and no fertilizer controls were added to bermudagrass plots held on the Mississippi State south farm to simulate "real-world" conditions and extend the baseline data gathered during the greenhouse trials previously conducted. A rainfall simulator was used to simulate precipitation events and following each rain event, runoff samples were collected for microbial analyses. Total Heterotrophic Plate Count (HPC) bacteria, antibiotic resistant bacteria (ARB), thermal-tolerant coliforms, enterococci, staphylococci, and *Clostridium perfringens* were investigated. Over a period of 60 days, 5 rain events were simulated using a portable rain applicator and results indicated that staphylococci, enterococci, and clostridia correlated well with manure application, corroborating the previous greenhouse study. Analysis of runoff concentration means demonstrated that in most cases litter application increased the presence of indicator microorganisms in runoff water. Traditional indicators such as thermal-tolerant and total coliforms performed poorly as fecal indicators relative to the other bacteria assayed in this study. No "frank" pathogens such as *Salmonella* or *Campylobacter* were detected in the applied litter or runoff. Chi square analysis of ARB indicated that litter application influenced the overall presence of antibiotic resistant bacteria, particularly with respect to polymixin B and aminoglycoside resistance. This study indicated that poultry litter land application can contribute to microbial runoff, however proper land and agronomic management practices can mitigate this.

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

Introduction

Fecally-derived wastes have been the recipient of public scrutiny for many years now. This may be due in part to the lack of government oversite and recent food-borne outbreaks possibly related to the land application of animal manure on or near agricultural lands intended for food crop growth. Though much research has been conduced in the area regarding over or under applying N and P from manure and their contribution to surface water contamination, very little has been conducted on the subject of manure-borne pathogenic or antibiotic resistant bacteria. Precautions must be taken to avoid runoff of these manure-borne nutrients as well as manure-borne microorganisms, which can survive land application and potentially be moved horizontally via runoff following rain events (Malik 2004; Thurston-Enriquez 2005).

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 grass 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 on small 84 sq ft plots located on the Mississippi State South Farm. Poultry litter (approximately 10 days old) was applied to the plots at two organic fertilizer rates of 250 lb N, and 50 lb P per acre, a high and a low rate respectively. Plots with inorganic fertilizer (250 lb N, and 50 lb P per acre) were used as control fertilizer; in addition plots with no fertilizer (organic or inorganic) applied were also used. Each plot was replicated in triplicate in a complete randomized block design. Rain was generated via the use of a constructed rain simulator and was operated from 27 to 50 mm/hr or the minimal volume and rate of rain necessary to generate runoff. A total of five rain events were simulated over a period of 70 days post application of litter, each lasting approximately 30 minutes. Field application took place in early May and rain events lasted 70 days after this.

Runoff water samples were collected from each plot following each rain event and were analyzed for microbial content including: Heterotrophic plate count bacteria (HPC), thermotolerant 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).

Results and Discussion

Overall, microbial runoff total from the litter-applied plots confirmed that C. perfringens would appear to be the best indicator organism of land applied poultry litter (Fig. 1-5). While traditional fecal indicators, such as themotolerant coliforms and enterococci performed poorly, staphylococci and clostridia appeared to be more suitable indicators, thus confirming previously reported literature for other forms of manure (Thurston-Enriquez 2005). The failure of traditional indicators may have been due to abnormally high background concentrations in the soil and hence runoff from controls and inorganic fertilizer application. The site is not pristine as it is surrounded by agricultural land and experiment stations housing horses, cattle, and poultry. In addition, it appeared that all monitored organisms increased slightly in concentration over the 70 day period (Fig. 2-4), while C. perfringens, an anaerobic spore forming bacteria, did not demonstrate any growth with the exception of one control sample on day 16. This was not due to growth, but most likely due to detection limits sensitivity. This may lend credence to the use of these types of bacteria as they demonstrate little willingness to amplify in the environment, however given organic matter and moisture E. coli and other fecal indicators have been shown to increase while in the environment (Rivera et al., 1988).

Staphylococci and enterococci isolates were analyzed for antibiogram and of these isolates, only enterococci isolates demonstrated increased resistance profiles. While this is not surprising, since Enterococcus is a genus ripe with resistance genes, it is concerning that much of this could end up as runoff. Much of the resistance was to the macrolide and aminoglycoside classes of antibiotics, and may represent the overall dominant presence of one clone or strain of Enterococcus in this particular applied poultry litter. It is important to note that microbial quality of poultry litter will differ from farm to farm, and from state to state, and as such antibiotic resistance genes will also differ. An issue associated with antibiotic resistance is that unlike pathogenic bacteria, which can be readily inactivated via compositing or storage, antibiotic resistant bacteria may be more hardy and able to survive the pressures of manure storage as demonstrated by this study. Salmonella and E. coli in the freshly collected poultry litter decreased by orders of magnitude (data not shown) with a simple storage step of approximately seven days, but antibiotic resistant bacteria in the same litter failed to be reduced significantly.

The issues associated with microbial runoff following poultry litter land application are not simple, due to the many different types of poultry litter applied throughout the country and lack of oversite. Simple steps can be taken to mitigate the runoff problem, by using grass buffers, monitoring applications close to rain events, and applying only what is needed for crop growth (Entry 2000; Stout 2005). Compounding the issues with fecal contamination of surface waters are the presence of antibiotic resistant organisms that when exposed to pathogens may or may not be able to transfer their resistance genes (Dzidic 2003). This is an issue that will only continue to draw the nation's ever watchful eye if not handled properly.

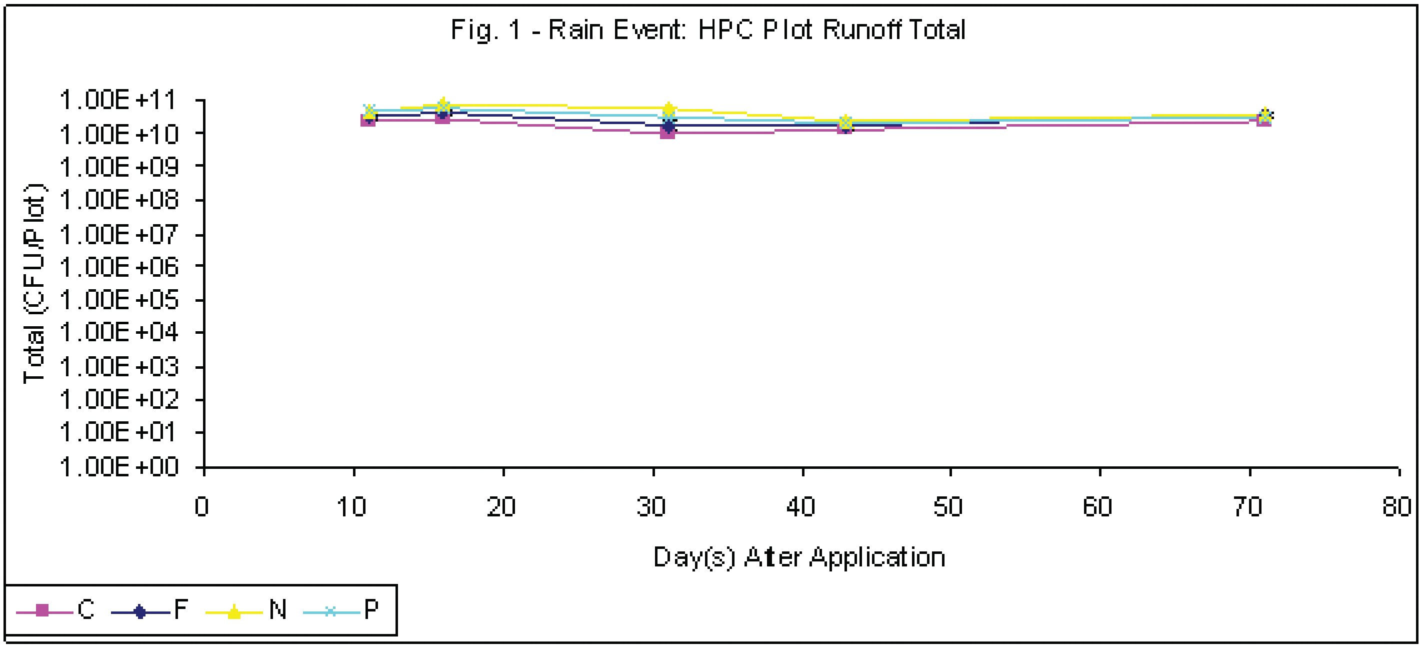


Fig. 1 - Rain Event: HPC Plot Runoff Total
Total (CFU/Plot)
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
Day(s) Ater Application
C
F
N
P

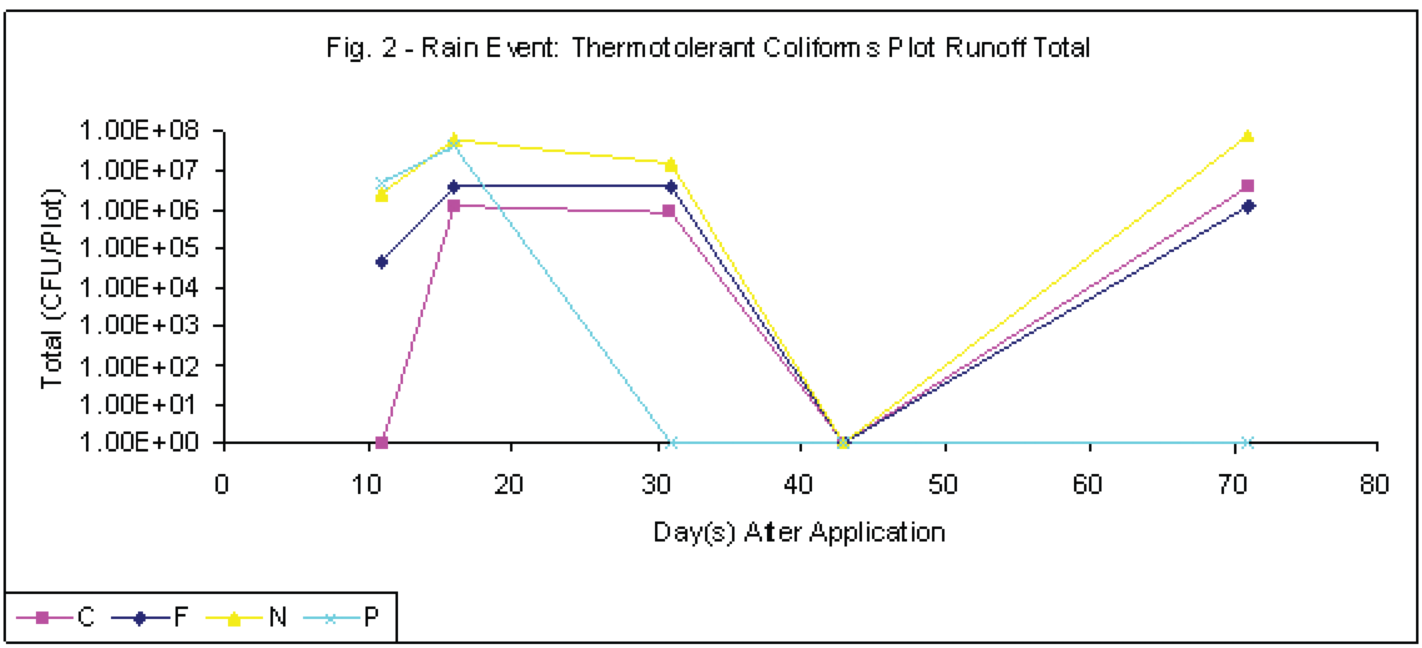


Fig. 2 - Rain Event: Thermotolerant Coliforms Plot Runoff Total
Total (CFU/Plot)
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
Day(s) Ater Application
C
F
N
P

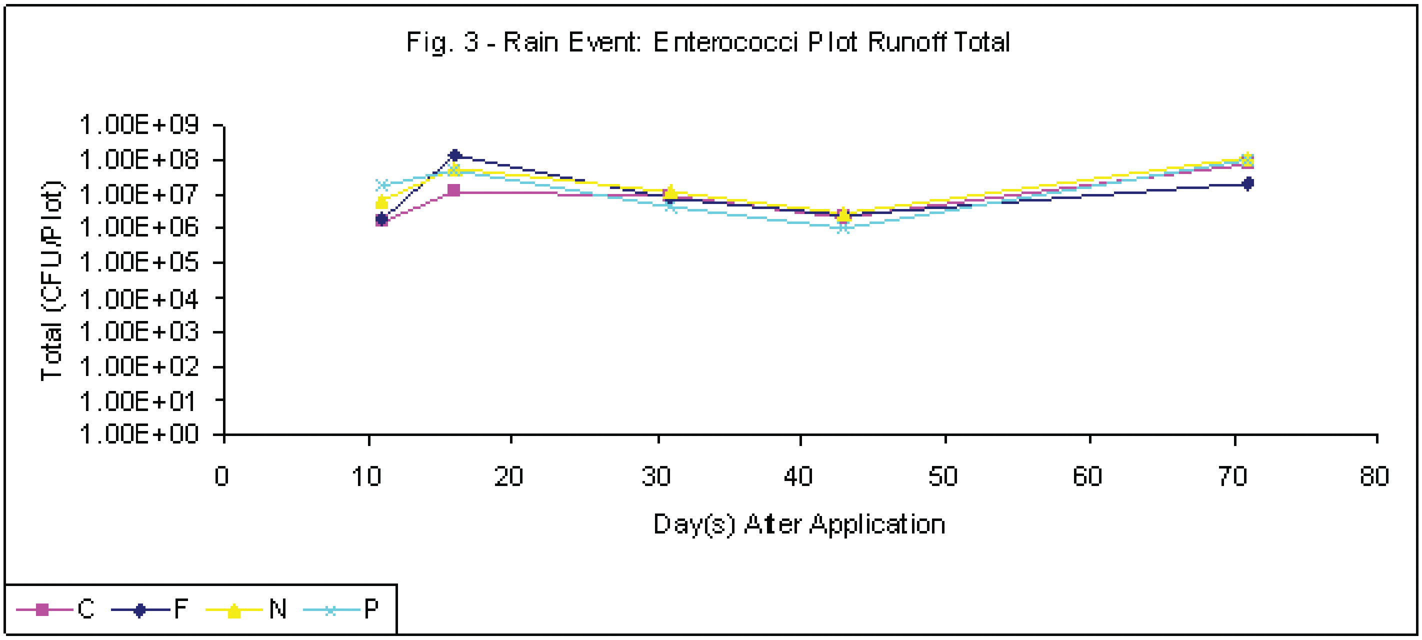


Fig. 3 - Rain Event: Enterococci Plot Runoff Total
Total (CFU/Plot)
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
Day(s) After Application
C
F
N
P

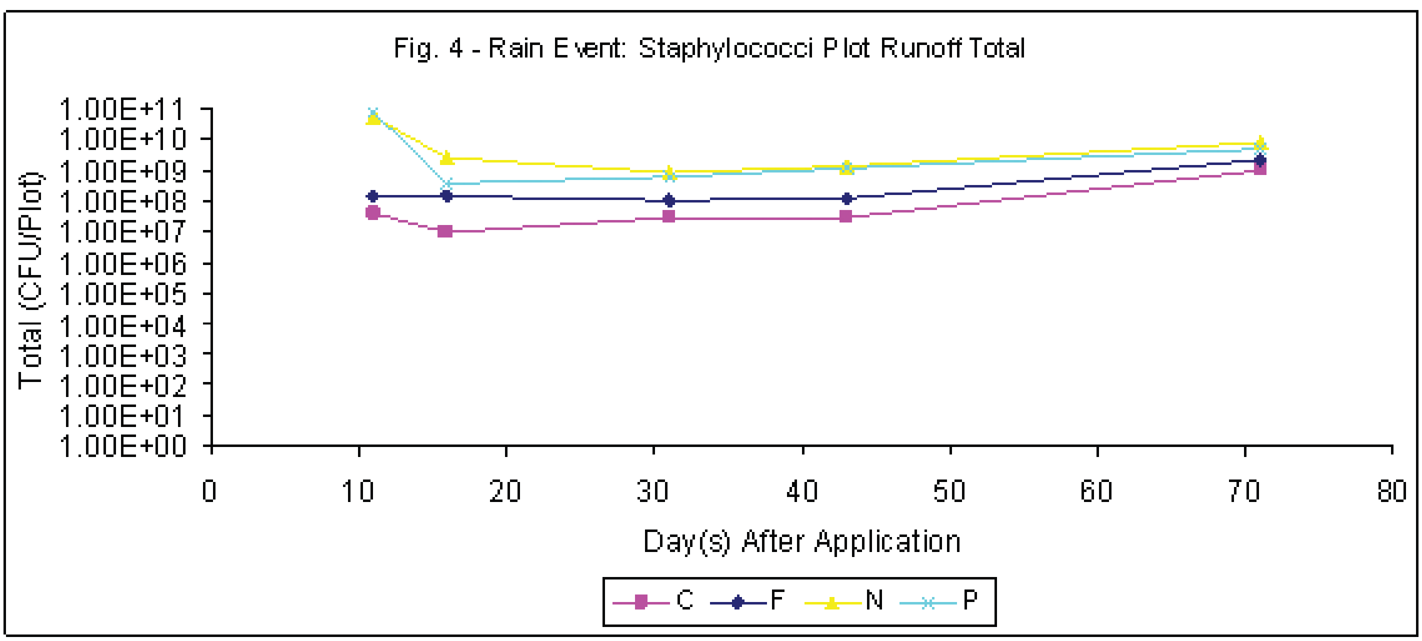
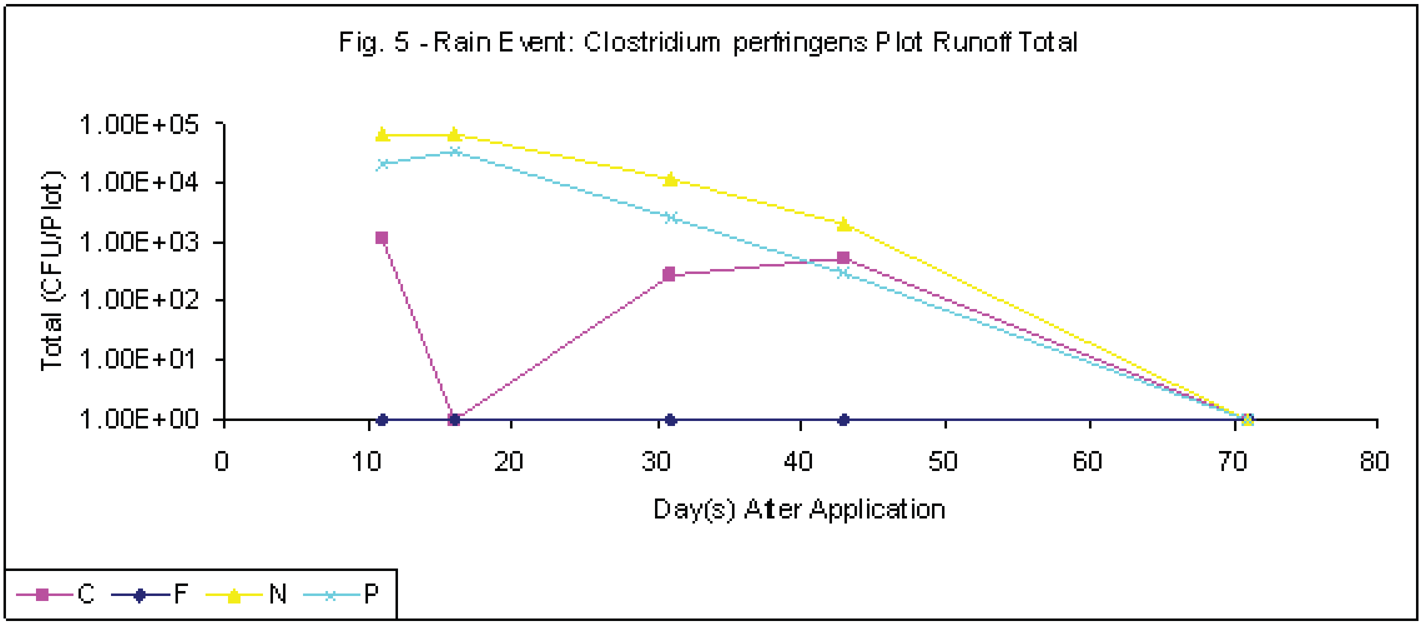


Fig. 4 - Rain Event: Staphylococci Plot Runoff Total
Total (CFU/Plot)
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
10
20
30
40
50
60
70
80
Day(s) After Application
C
F
N
P



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.

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.

Rivera, S.C., T.C. Hazen, and G.A. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. Applied and Environmental Microbiology 54:513-517.

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.