

**Title: Fecal Coliform Concentration in Runoff from Fields with Applied Dairy Manure**

**Project Number:**

**Start Date: 3/01/02**

**End Date: 3/01/03 (extension approved)**

**Research Category: NPP, WQL, AG**

**Descriptors: fecal coliform bacteria, *E. coli*, non-point source pollution, water quality, dairy manure**

**Lead Institution: Louisiana State University Agricultural Center (BAE Department)**

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**Number of Students supported: 2 undergraduate students; 1 M.S. student**

**Problem and Research Objectives**

Wastes produced in livestock production and processing facilities are increasingly applied to agricultural soils for either disposal and/or nutrient recycling (Coyne et al, 1995). Once manure is land applied, it becomes a potential agricultural non-point source of pollution. Pasturing operations also contribute to this type of pollution (Moore et al., 1989). One of the main concerns with land application of animal manure is that bacterial pathogens will reach groundwater and surface water via runoff during or after a storm event (Stoddard et al, 1998). Infectious diseases of microbiological etiology, originating in man and other animals, can be transmitted through waters that receive animal wastes. Thus, human and livestock exposure to surface or groundwater contaminated with fecal bacteria is an important water quality concern (Stoddard et al, 1998).

Cattle grazing on rangelands and pastures may account for a significant amount of non-point source water pollution. Further research is needed to determine the extent to which dairy manure on pasture impacts water quality. Studies comparing methods of waste application

indicate that the effect of grazing cattle on water quality may differ from that of land applied manure.

Viable fecal coliform (FC) bacteria have been cultured from fecal deposits after extended periods of time, even as long as one year if conditions are favorable (Thelin and Gifford, 1983). Buckhouse and Gifford (1976) in Kress and Gifford (1984) found that fecal coliforms persist in cow feces at least seven weeks under hot, dry summer range conditions. Thus, fecal deposits are capable of providing a long-term source of potential pollution to surface water (Thelin and Gifford, 1983). The fecal deposit appears to act as a protective medium for the bacteria within by forming a crust, thus decreasing interaction of the bacteria with the soil and atmosphere (Stoddard et al, 1998). Thelin and Gifford (1983) have found that a large population of fecal coliforms still exists in a fecal deposit long after the deposit has been thoroughly dried.

Not only have coliform bacteria been found to survive outside of the intestinal tract, but they also have been found to have exhibit initial regrowth in certain conditions. Moist conditions, mild temperatures, and manure crusting are believed to contribute to regrowth (Stoddard et al, 1998). Organic nutrients present within the feces may also provide a favorable environment for growth (Van Donsel et al., 1967). Additionally, fecal bacteria are able to obtain nutrients associated with the sediment particles (Davies et al, 1995). An initial rise in coliform bacteria was noticed in many studies both in soil and in water (Dewedar and Baghat, 1995). Van Donsel et al. (1967) noted evidence of soil coliform growth following rainfall. Although bacteria have been noted to remain viable for an extended period of time when protected within a fecal deposit, the hydrophobic properties of the dried outer crust may prevent bacterial interaction of with rain (Stoddard et al, 1998).

Bacteriological water quality is determined by examining water samples for the presence of indicator organisms. Measurement of the quantity of fecal coliform bacteria is one of the most commonly used methods to establish the quality of natural waters (Valiela et al, 1991). The fecal coliform group is indicative of organisms originating in the intestinal tract of humans and other animals (Thomann and Mueller, 1987) although other sources may exist (Auer and Niehaus, 1993). Fecal coliforms are a thermotolerant group that decline in natural environments. Procedures for routine measurements have been standardized (Valiela et al, 1991), however, the test methods are both labor and materials intensive, and require precise control of laboratory conditions and a high degree of technical skill to perform and interpret results. Such methods include the Membrane Filter (MF) and Multiple Tube Fermentation tests.

A relatively new method, the QuantiTray-Colilert system (developed by IDEXX Laboratories, Inc.), was designed to measure *E. coli* and total coliforms (TC) in drinking water simultaneously (Elmund et al, 1999). This method is much simpler requiring less manipulation, and thus has less chance for human error. The QuantiTray system is very easy to inoculate. Time studies indicate that the QuantiTray system requires significantly less time per sample for setup, reading, and recording of results (Budnick et al., 1996).

The Colilert reagent is a defined substrate MUG-based media for detecting total coliforms and *E. coli*. Per the IDEXX method, 100-ml water samples (diluted with sterile water, if necessary) are mixed with pre-measured packets of Colilert reagent in polystyrene bottles and mixed via rigorous shaking. The mixture is then poured into a QuantiTray, a sterile plastic disposable panel consisting of 96 discrete “wells.” The QuantiTray is then mechanically sealed to distribute the sample mixture into all of the wells.

This system provides a Most Probable Numbers (MPN) result based on the presence or absence of yellow color and fluorescence in the individual wells after incubation at 35°C (Fricker et al., 1997). Quantitative results are available within 24 hours. The presence of yellow pigmentation in a well is considered a positive reaction indicating the presence of total coliforms. QuantiTray wells showing no color are considered negative for total coliforms. Wells that fluoresce under UV light are considered positive for the presence of *E. coli*. The positive wells on each tray are counted and compared to a reference table that gives corresponding MPN count of TC or *E. coli* per 100 mL.

One drawback of the QuantiTray method is that there is currently no available procedure for quantifying fecal coliforms. To determine whether or not the QuantiTray system can accurately quantify fecal coliforms using Colilert reagent, the incubation temperature will be raised to the 44.5 °C to discourage growth on non-fecal coliforms. Subsequently, the recovery of fecal coliforms from the QuantiTray method at this higher temperature will be directly compared to the recovery of fecal coliforms from the MF technique.

Therefore, the purpose of the study was to quantify microbial pollutant transport in surface runoff over manure-amended dairy pastures following rainfall. The specific objectives are as follows:

1. To determine the fecal coliform (FC) concentrations in surface runoff after simulated rainfall from field pasture plots as affected by different methods of manure application and recurrent rainfall and compared to control plots; and
2. To determine whether the IDEXX QuantiTray method is an accurate and reliable method for enumerating fecal coliforms as compared to an approved standard method (Membrane Filtration).

## **Methodology**

The experiment took place at Southeast Research Station in Franklinton, Louisiana. Cattle did not graze at the study site during the experiment or for the prior six years.. Nine field plots were constructed in a 3-row by 3-column layout. Manure application as deposited by cattle (Treatment A) was compared to land application of manure as fertilizer (Treatment B). Application of inorganic fertilizer was used as the control treatment (Treatment C). Each treatment was replicated on three plots in a randomized design. Fresh manure was collected from the feed stall prior to field plot application. Simulated rainfalls were conducted on plots within several hours after initial manure application and runoff samples were collected. Subsequent rainfall simulations were conducted approximately 2, 7 and 14 days after the initial manure application. This sequence was repeated a total of three times. Fecal coliform analyses were performed using Membrane Filtration (APHA, 1995), and the QuantiTray method by IDEXX at incubation temperatures of 35 and 44.5 °C.

## **Principal Findings and Significance**

The FC concentrations in the surface runoff from the field plots after simulated rainfall Series I are shown in Figure 1. FC counts increased slightly from Day 0 to Day 2 for both the manure treatments (A and B), indicating initial regrowth of the bacteria, and then decreased with time. In Series I, the mean FC concentration for Day 0 runoff from the control plots was 285 cfu/100 mL, near the standard for primary contact recreation of a mean of 200 and a maximum of 400 cfu/100 mL. The mean FC counts for Day 0 runoff from Treatment A and Treatment B were approximately 8,000 and 120,000 cfu/100 mL, 20 and 300 times the primary standard. After 5 rainfall simulations over 30 days, the mean FC concentrations in runoff decreased to

approximately 2,000 and 4,000 cfu/100 mL for Treatments A and B, respectively. Similar results were obtained for Series II and III rainfalls.

To compare the impact of the manure treatments, the FC loading was calculated as the FC concentration in the surface runoff multiplied by the volume of runoff produced. Mean FC loading values are shown for Series I in Figure 2. Using data over the three rainfall series, the natural log of the FC loading for Treatment B ( $m = 15.9$ ,  $sd = 1.9$ ) was significantly greater than Treatment A ( $m = 13.7$ ,  $sd = 1.9$ ), and both manure treatments were significantly greater than the control, Treatment C ( $m = 9.8$ ,  $sd = 1.8$ ). Similar results were obtained when data for individual series were tested. These results indicate that distributed manure application releases a greater FC loading to surface water than simulated natural fecal deposition, for the small field plots used in this study.

Fecal coliform enumeration using the QuantiTray method was significantly correlated with that of membrane filtration for both manure treatments, but not for the control. A strong correlation was found for Treatment B ( $\rho = 0.747$ ,  $p < 0.001$ ) and a mild correlation was found for Treatment A ( $\rho = 0.497$ ,  $p < 0.001$ ).

## **DISCUSSION**

The first rainfall event of each Series was representative of a worst-case scenario in which waste application was followed after only a brief interval by a moderate intensity rain. The results of this experiment indicated that at a loading of 1.5 cows/ha, the potential exists for fecal coliform concentration in runoff to exceed the standard for primary contact recreation. However, these concentrations were obtained from runoff that had traversed a very small distance with no buffer or filter zone.

The results of this study indicate that fecal coliform concentrations in runoff from pasture representative of cattle grazing were significantly lower than those obtained from land applied manure. Fecal coliform concentrations and loadings in runoff from plots with Treatment B (scattered manure) were significantly higher than from those plots with Treatment A (manure deposits) in all three Series. These findings indicate that the manure crusting effect may play a role in reducing the potential number of fecal coliforms transported in runoff to surface waters. While the external crust of the manure deposit may provide a shelter for bacteria from environmental factors, its hydrophobic properties also prevent bacterial contact with runoff water. Another rationale for this occurrence is that the available surface area of manure is greater in Treatment B than in Treatment A, thus increasing the potential contact between raindrops and manure.

The presence of fecal coliforms in the runoff from plots with Treatment C could be due attributed to contamination by wildlife and/or the presence of thermotolerant soil coliforms such as *Klebsiella* (Moore et al., 1989). In many studies, little difference is seen between areas used as pastures and control areas where manure has not been spread (Kunkle, 1970; Robbins et al., 1972; Doran and Linn, 1979;).

The results of this study indicate that IDEXX may be an acceptable alternative to the standard method of Membrane Filtration for enumerating fecal coliforms. Although the IDEXX medium was formulated for total coliform enumeration at 35°C, the results of this study indicate that when incubated at 44.5°C, counts are significantly correlated with those of Membrane Filtration for both manure Treatments. The false positives obtained during both membrane filtration and the QuantiTray method may explain why a correlation was not established for the control.

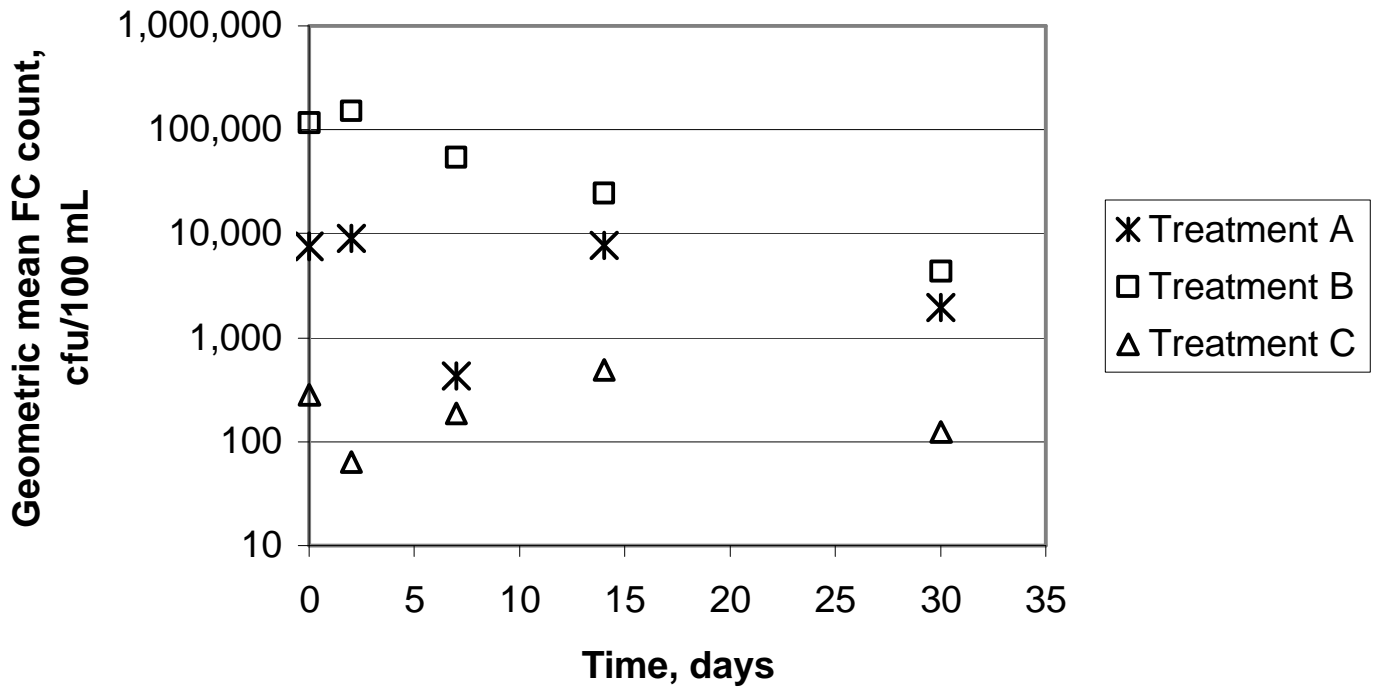


Figure 1: Mean FC counts in surface water from field plots after simulated rainfall Series I.

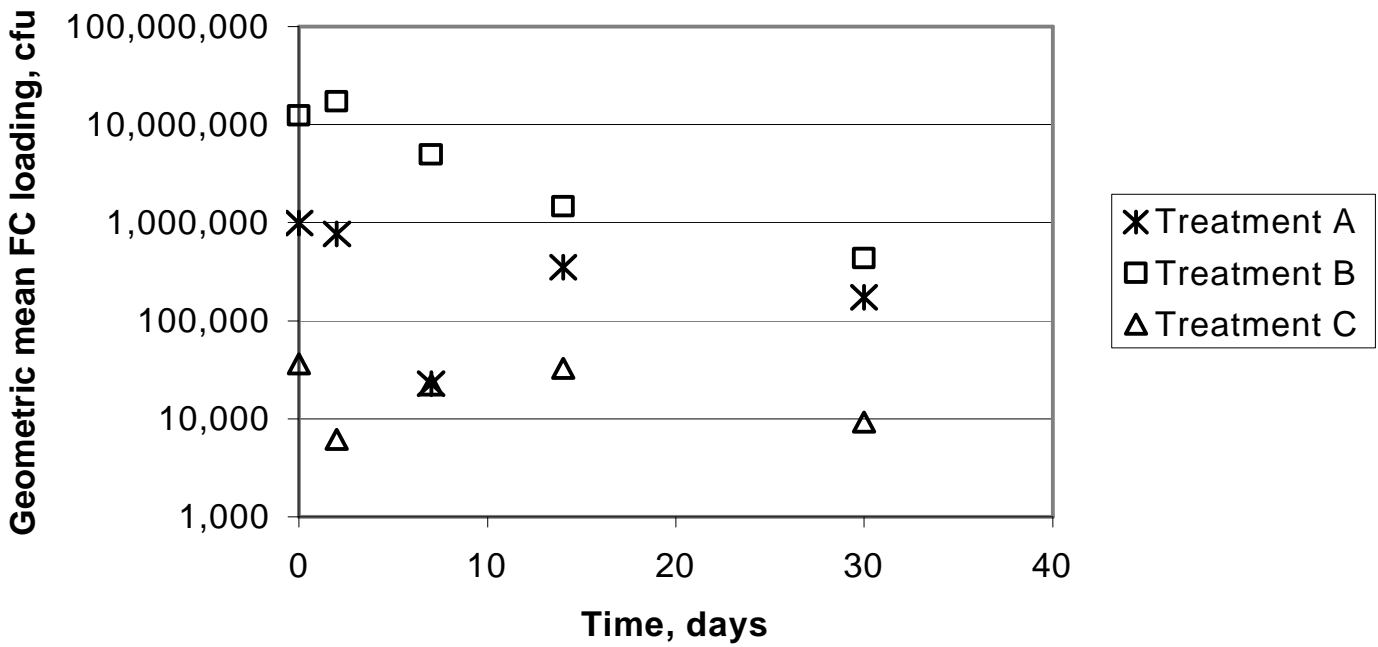


Figure 2: Mean FC loading in surface water from field plots after simulated rainfall Series I.

## References

- American Public Health Association. 1995. Standard methods for the examination of water and wastewater, 19th ed. APHA, Washington, DC.
- Auer, M.T., and S.L. Niehaus. 1993. Modeling fecal coliform bacteria—I. Field and laboratory determination of loss kinetics. *Wat. Res.* 27(4):693-701.
- Budnick, G.E., R.T. Howard, and D.R. Mayo. 1996. Evaluation of enterolert for enumeration of enterococci in recreational waters. *Applied and Environmental Microbiology.* 62(10):3881-3884.
- Buckhouse, J.C. and G.F. Gifford. 1976. Water quality implications of grazing on a semiarid watershed in southeastern Utah. *J. Range Manage.* 29:109-113.
- Coyne, M.S., R.A. Gilfillen, R.W. Rhodes, and R.L. Blevins. 1995. Soil and fecal coliform trapping by grass filter strips during simulated rain. *J. Soil and Water Cons.* 50(4):405-408.
- Davies, C.M., J.A.H. Long, M Donald, and N.J. Ashbolt. 1995. Survival of fecal microorganisms in marine and freshwater sediments. *Applied and Environmental Microbiology.* 61(5):1888-1896.
- Dewedar, A. and M. Baghat. 1995. Fate of fecal coliform bacteria in a wastewater retention reservoir containing *Lemna gibba* l. *Wat. Res.* 29(11):2598-2600.
- Doran, J.W., and D.M. Linn. 1979. Bacteriological quality of runoff water from pastureland. *Appl. Environ. Microbiol.* 37: 985-991.
- Elmund, G.K., M.J. Allen, and E.W. Rice. 1999. Comparison of *Escherichia coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environment Research.* 71(3):332-339.
- Fricker, E.J., K.S. Illingworth, and C.R. Fricker. 1997. Use of two formulations of Colilert and QuantiTray for assessment of the bacteriological quality of water. *Wat. Res.* 31(10): 2495-2499.
- Kress, M., and G.F. Gifford. 1984. Fecal coliform release from cattle fecal deposits. *Water Resources Bulletin.* 20(1):61-66.
- Kunkle, S.H. 1970. Sources and transport of bacterial indicators in rural streams. p. 105-132. IN *Proc. Symp. on Interdisciplinary aspects of watershed management.* ASCE, New York.
- Moore, J.A., J.D. Smyth, E.S. Baker, J.R. Miner, and D.C. Moffitt. 1989. Modeling bacteria movement in livestock manure systems. *Transactions of the ASAE.* 32(3):1049-1053.
- Robbins, J.W.D., D.H. Howells, and C.J. Kriz. 1972. Stream pollution from animal production units. *J. Water Pollut. Control Fed.* 44: 1536 - 1544.

Stoddard, C.S., M.S. Coyne, and J.H. Grove. 1998. Fecal bacteria survival and infiltration through a shallow agricultural soil: timing and tillage effects. *J. Environ. Qual.* 27:1516-1523.

Thelin R., and G.F. Gifford. 1983. Fecal coliform release patterns from fecal material of cattle. *J. Environ. Qual.* 12(1):57-63.

Thomann R.V. and J.A. Mueller. 1987. *Principles of Surface Water Quality Modeling and Control.* Harper & Row, New York.

Valiela, I., M. Alber, and M. LaMontagne. 1991. Fecal coliform loadings and stocks in Buttermilk Bay, Massachusetts, USA, and management implications. *Environmental Management.* 15(5):659-674.

Van Donsel, D.J., E.E. Geldreich, and N.A. Clarke. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. *Applied Microbiology.* 15(6):1362-1370.