# Survival of Food-borne Pathogens in Soil under Field Conditions

**A USDA CSREES NRI Project (2007-3512-18243)**

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## Abstract

- *Escherichia coli* O157:H7 (*E. coli* O157) and *Salmonella enterica* serovar Newport (*Salmonella* Newport) are zoonotic pathogens imposing serious public health concerns. The *E. coli* O157 outbreak in the fall of 2006 via contaminated spinach is a recent example, whereas *Salmonella* Newport currently ranks third by CDC in causing human intestinal infections. Both pathogens have established reservoirs in dairy cattle. As infected animals shed the organisms in manure and manure is subsequently spread on agricultural land, the pathogens may be transmitted to plants, other animals or humans, or transported into waters. However, information is lacking on how the organisms may survive and move in the post-shed environment. The purpose of this project is to enhance the understanding of the fate and impact of these pathogens in agroecosystems and to contribute to the development of science-based and farm-applicable management strategies for their control. Specific objectives are: (i) Assess the survival characteristics of *E. coli* O157 and *Salmonella* Newport in manure and manured soils under laboratory conditions, (ii) Determine the persistence of the organisms in different manure handling/storage and cropping systems on farms, (iii) Investigate the transport behavior and factors affecting movement of the organisms from manured soils to waters through leaching studies and on-farm investigations. Results reported here are from preliminary trials and will be used to refine experimental protocols for the main study beginning in spring 2008.

## Background

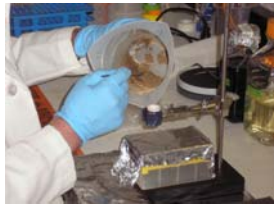
- Recent, high-profile outbreaks of food-borne disease associated with ground beef, fast food outlets, spinach and other food sources have been ascribed to zoonotic pathogens (CDC, 2006).
- *Salmonella* Newport and *E. coli* O157:H7 have established reservoirs in dairy herds.
- Bacteria such as *Salmonella* and *E. coli* can be transmitted from manured soil or contaminated waters into the food chain.
- To date, little data on these microorganisms' survival characteristics and behavior in the soil and water environment.
- Sentinel chambers ideal for this type of investigation, design able to retain potentially dangerous organisms inside chambers while allowing equilibration with natural environmental conditions.

## Objectives

- Using replicated sets of sentinel chambers embedded in the field, follow time-course of *Salmonella* Newport and *E. coli* O157 survival in soil under natural conditions.
- Adapt procedures as problems arise and are resolved so as to prepare for the main study.

## Materials and Methods

- Sentinel chambers adapted from Jenkins et al. (2002), fabricated from PVC pipe, 5 $cm^3$ volume, ends sealed by selectively-permeable 0.22 µm nitrocellulose membranes.
- Pure cultures of *Salmonella* Newport or *E. coli* O157 mixed with moist, sieved field soil aggregates amended with dairy manure.
- 2.5 – 3 g soil-manure mixture to fill each sentinel chamber. Three replicated treated sentinels and one without bacteria inoculation to monitor soil moisture inserted into sample modules.
- Modules had nylon mesh covers top and bottom to prevent coarse soil debris from puncturing membranes.
- Sample modules installed in November 2007, samplers covered by 5 cm soil.
- Samples removed periodically to monitor moisture content over time and perform population counts.
- Diluted extracts plated on selective media, (XLD agar for *Salmonella* Newport and sorbitol-MacConkey agar for *E. coli* O157).



Preparing sentinel chambers

Sentinel module before/after field installation

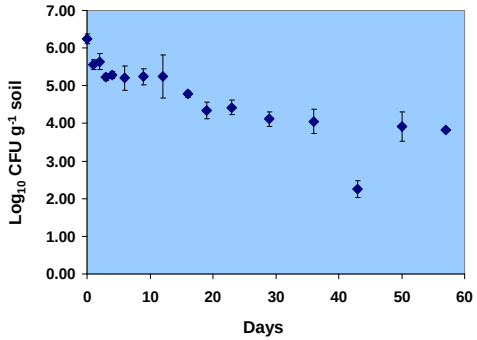
Salmonella-positive plate under darkfield counter

## Results and Discussion

### *Salmonella* Newport Population

- A consistent slow decline through day 57, total count declined by approx. 100-fold.
- A relatively rapid die-off between day 16 and 23 corresponding to a period of low rainfall.
- Unclear why the anomalously low plate counts on day 43; all plates had fewer *Salmonella* CFU than samples collected before or after.

***Salmonella* Newport survival in soil system over 8 weeks**



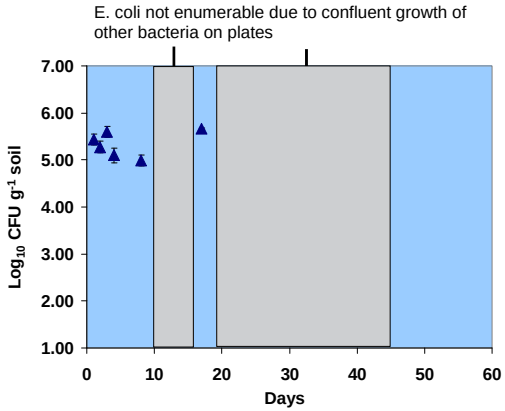

### *E. coli* O157 Population

- No distinct trend in introduced *E. coli* O157 die-off through first 3 weeks of study.
- At many sampling times, *E. coli* enumeration was difficult because of unknown microorganisms overwhelming any *E. coli* O157 colonies.

### Methodology issue/potential resolutions

- (1) *E. coli* strain used in this trial was Shiga-toxin negative, has been shown to be more difficult to culture than Shiga-positive strain. Possible to switch to Shiga-positive. Other growth media are also being tested.
- (2) Introduced *E. coli* in soil system appears to be readily out-competed by native soil microorganisms. Sterilizing soil-manure mixture before inoculation would eliminate competition but deviate from natural conditions. Issue not yet resolved.

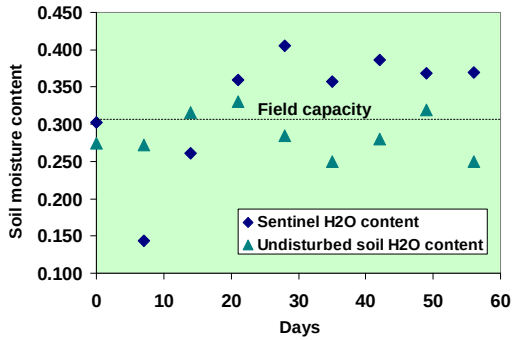
***E. coli* O157:H7 survival in soil over 7 weeks**




### Sentinel Equilibration with Environmental Conditions

- Inoculated soil-manure mixture used to fill sentinels appeared to dry out more quickly under dry soil conditions and retain moisture longer under wet conditions than surrounding soil.
- Using straw mulch or covering with sod may allow better equilibration of sentinels with natural soil conditions.

**Sentinel soil moisture content compared to undisturbed soil**




## Conclusions

- *Salmonella* Newport declined at a slow rate through 8 wks of early winter weather conditions. While they tend to not proliferate in the soil, once introduced in fecal material they have been shown to survive for long periods in the agroecosystem.
- *E. coli* O157 was difficult to work with due to problems with culturing and competition with other microorganisms. Alternative growth media are being tested and changes in the methods are under consideration.
- Modifying the sentinel system design should allow a better representation of natural field conditions inside the chambers.

## References


- Centers for Disease Control. 2006. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, United States, 2005. Morbid. Mortal. Weekly Rept. 55:392-395. Online at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5514a2.htm.
- Jenkins, M.B., D.D. Bowman, E.A. Fogarty, and W.C. Ghiorse. 2002. *Cryptosporidium parvum* oocysts inactivation in three soil types at various temperatures and water potentials. Soil Biol. Biochem. 34:1101-1109.