Agronomy Notes Vol. 30 No. 3

Escherichia coli Pathogen O157:H7 Does Not Survive Longer In Soil Than A Nonpathogenic Fecal Coliform

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Introduction

Survival rates for individual types of fecal organisms are quite different. Although some pathogens may persist as long as 5 years in soil, most fecal pathogens from human and animal waste usually die very quickly. Two to three months is sufficient in most cases to reduce pathogens to negligible numbers once they have been excreted or land-applied in animal wastes. It is expensive and time-consuming to test for individual pathogens. Consequently, nonpathogenic fecal indicator bacteria, which are easily and inexpensively detected, are often used to study pathogen survival in soil and water. Current methods for rapidly detecting fecal indicator bacteria use the capacity of fecal coliforms (e.g. *Escherichia coli*) to metabolize a fluorescent indicator compound, 4-methylumbelliferyl ß-D-glucuronide (MUG) as evidence for fecal contamination.

Some fecal coliforms are unable to hydrolyze MUG, which means they give false negative responses to this quick test. Among the *E. coli* strains with a MUG negative response is the virulent enterohemorrhagic (EHEC) pathogen designated as O157:H7. Strain O157:H7 is widely spread and has been found in birds, cattle, deer, dogs, horses, sheep, and swine. It is transmitted via the fecal-oral route and fewer than 100 bacteria can cause illness. O157:H7 is the predominant EHEC strain in the U.S. and has caused several deaths by kidney failure due to contaminated drinking water, food, and recreational swimming pools. The very young and elderly are the most susceptible groups at risk from this pathogen, although all ages can be affected.

The consequences of virulent fecal coliforms persisting in the environment are serious, but comparative studies relating the survival in soil of pathogenic *E. coli* strains, such as O157:H7, to typical nonvirulent indicator fecal coliforms are lacking. This study was therefore conducted to compare the mortality of *E. coli* O157:H7 and a nonvirulent *E. coli* strain in two Kentucky soils.

Methods

The experiment design was a 2 x 2 factorial with replication, which compared two soils and two bacteria strains. Pope silt loam and Zanesville silt loam, which differ in physical and chemical properties, were used in the study (Table 1). *Escherichia coli* strain O157:H7 and a nonvirulent *E. coli* strain were prepared by growing cultures in a nutrient broth. The cells were harvested, washed, resuspended, and then added to air dried, nonsterile, soil. The final soil water content was 20% by weight. There were no detectable fecal or total coliforms in the soils before adding the bacteria. The inoculated soil was subdivided among polyethylene bags and stored at 77 ˚F (25 ˚C) for the duration of the experiment.

Escherichia coli were enumerated from three replicate samples every week for 8 weeks using a most probable number method. Ten grams of soil from each sample were serially diluted in 10-fold steps until no bacteria remained. Culture tubes containing media that revealed activity by *E. coli* were inoculated from each soil and dilution. *Escherichia coli* presence was identified after incubation at 95 ˚F (35 ˚C) for 48 h and their numbers were determined based on the dilutions at which *E. coli* were no longer present.

Escherichia coli death was modeled by the linear equation:

$$\log_{10} A = -kt + \text{constant}$$

where A = the *E. coli* population, k = the death rate, and t = time. The data are reported as the half life ($t_{1/2}$), the time required for half the *E. coli* population to disappear. A simple regression was performed on this linear equation to calculate the best-fit for the data and determine whether more complex models were required to adequately describe *E. coli* mortality.

Results and Discussion

Figure 1 shows the *E. coli* population as a function of days of incubation. Both strains had similar survival patterns in the individual soils. However, there were big differences in the survival pattern between soils. Bacteria populations typically disappeared more rapidly in Pope soil than Zanesville soil. Exchangeable bases, soil organic matter, and total nitrogen, which have been associated with increased fecal bacteria survival in soil, were generally greater in Pope soil than Zanesville soil. However, in the first week, die-off in Pope soil was apparent, while both strains evidently regrew in Zanesville soil. Although both soils had silt loam textures, Pope soil had twice as much clay as Zanesville soil (Table 1). One reason that *E. coli* death began immediately in Pope soil was probably because the higher clay content created greater water stress than in Zanesville soil, even though both soils had the same initial gravimetric water content.

There was not a significant difference in the half lives of the two strains in either soil. Both strains died off faster in Pope silt loam than Zanesville silt loam. The half life of O157:H7 in Pope silt loam was 1.8 days and that of the nonvirulent *E. coli* strain was 2.2 days (Table 2). In Zanesville silt loam, the half life of O157:H7 was 2.7 days and that of the nonvirulent strain was 3.3 days. These mortality rates are somewhat higher than have been observed in labortory and field studies of survival and probably reflect the use of laboratory cultures rather than fresh animal wastes as the source of *E. coli.* The *E. coli* in dairy manure applied to no-till or chisel disk soils in spring and fall, for example, have half lives of 7 to 8 days.

Although *E. coli* death in soil could be described as a single stage process, the reduction in bacteria numbers was better described when it was separated into two stages. The mortality rate constants derived from a two-stage model indicated that the initial mortality rates depended on soil type whereas the subsequent mortality rates depended on the *E. coli* strain. The initial mortality rates compared to the subsequent mortality rates indicated that soil type had a greater effect on mortality than the type of *E. coli* strain.

Based on the calculated mortality rates, between 40 and 60 days would be required for 99.9% of the added *E. coli* to be eliminated from either soil under these soil conditions. If the soil environment were drier, we would anticipate much more rapid mortality rates from both bacterial strains. Conversely,

the literature suggests that if soil conditions were wetter and cooler than we have described, mortality rates would decline and the bacteria in soil would persist longer. However, the comparative mortality rates we have described for these *E. coli strains* should be unaffected by changes in soil environmental conditions.

Conclusions

It is generally accepted that fecal indicator microorganisms are affected in the same manner as pathogens in soil. In most studies they seem to survive longer than pathogens. In this study, the nonvirulent, MUG positive *E. coli* strain consistently had longer half lives and therefore survived in higher numbers than the pathogen O157:H7. However, the differences were not statistically significant. This suggests that the pathogen O157:H7 is unlikely to predominate in soil environments and increase the risk that fecal contamination of an environment is undetected. Rapid tests for *E. coli* detection in soil and conservative preventive measures after their detection should be adequate to minimize health risks due to fecal contamination of soil and groundwater, even though some potential *E. coli* strains, such as O157:H7, elude detection by these methods.

Although the overall regression coefficients from a single stage model were relatively high, the mortality rate constants derived from the model appeared to underestimate *E. coli* persistence in the two soils tested. As a matter of public health, a more conservative approach is to use a two-stage model, which seems to be a better predictor of the survival of *E. coli* in soil than a single stage model.

Table 1. Soil physical and chemical characteristics prior to *E. coli* addition.

Characteristic	Pope silt loam	Zanesville silt loam
Texture (%)		
Clay	25	12
Silt	59	67
Sand	16	21
pH	5.6	5.5
Mehlich III extractable (mg kg^{-1})		
Ca	1379	980
Mg	155	74
K	85	44
P	21	14
Zn	13	2
Total N (mg kg^{-1})	1510	1000
Soil organic matter (%)	2.8	1.7

Table 2. Half lives of *E. coli* strains in Pope and Zanesville silt loams.

		Half lives (days)		
		Single stage model	Two stage model	
Soil	Strain		Stage 1	Stage 2
Pope	Nonpathogen	2.2 (0.89)†	1.4 (0.94)	5.0 (0.96)
	O157:H7	1.8 (0.93)	1.2 (0.99)	3.9 (0.99)
Zanesville	Nonpathogen	3.3 (0.90)	1.8 (0.97)	6.0 (0.95)
	O157:H7	2.7 (0.93)	2.0 (0.87)	4.3 (0.94)

†The regression coefficient for goodness of fit, r^2, is noted in parentheses after each half life.

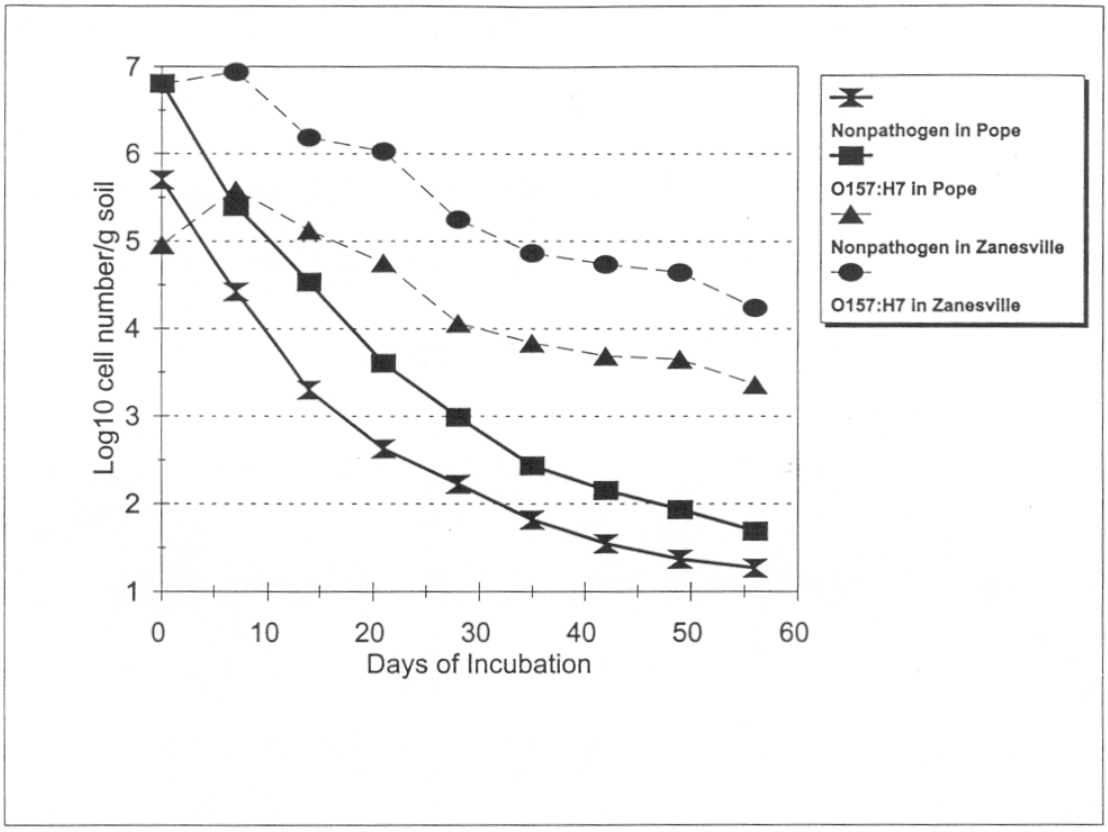

Figure 1. Mortality of *E. coli* strains in two soils with different physical and chemical characteristics