Exploration of the Impact of Application Intervals for the Use of Raw Animal Manure as a Soil Amendment, on Tomato Contamination

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Background

- Manure is commonly used as a fertilizer in vegetable production fields on both conventional and organic farms. Application of untreated biological soil amendments of animal origin may represent a potential risk for fresh produce of contamination with enteric pathogenic bacteria.
- FDA is conducting a risk assessment and, in collaboration with the U.S. Department of Agriculture and other stakeholders, is undertaking critical research to strengthen scientific support for any future proposal regarding the appropriate time interval(s) between application of biological soil amendments and harvest. The present work contributes to that research.

Objectives

1. To examine the survival of generic *E. coli* cocktail strains applied to soil amended with manure for 9 months and potential transfer to tomatoes for 12 months
2. To compare survival of *E.coli* on soil amended with different animal manure types: horse, cattle, goat, chicken litter and no manure (control)

Methods

Experimental design and manure treatment

- The field trial was carried out in 2013-2015 in the University of California Davis Vegetative Crop. Field plot (1x2 m) amended with chicken litter (4 lbs.), horse (5 lbs), cattle (5 lbs), goat manures (5 lbs), and no treatments were inoculated with 1L of bacterial fecal slurry.
- Three strains of indicator *E. coli* resistant to rifampicin were inoculated in high (10^7 CFU/ml) or low (10^4 CFU/ml) inoculum.

Sampling procedures

- Soil samples from 44 plots including negative control plots (n=4) were collected on day 0, 1, 3, 7, 14, 28, and every month until 9 months
- Four replications were applied all manure types with low and high inoculum
- Five tomato fruits per plot were harvested from month 4 to 7.
- Direct plate count and most probable number (MPN) were used to quantify bacterial concentration.

Figure 1: Low and high inoculum were separately transferred to a backpack sprayer

Figure 2: Horse, cattle, goat, chicken litter manure, and no manure field plot were inoculated with indicator *E. coli* and controls were set up at the end of the field.

Results and Discussion

- In the present study, an average of a 7 log reduction of inoculated *E. coli* was observed, across all types of manure after 120 days from the time of manure application.
- The generic *E. coli* populations survived longest in untreated chicken litter followed by horse, cattle and goat manure.
- *E. coli* populations increased after heavy rains by an average of 5 to 6 log CFU in both high and low inoculum plots.

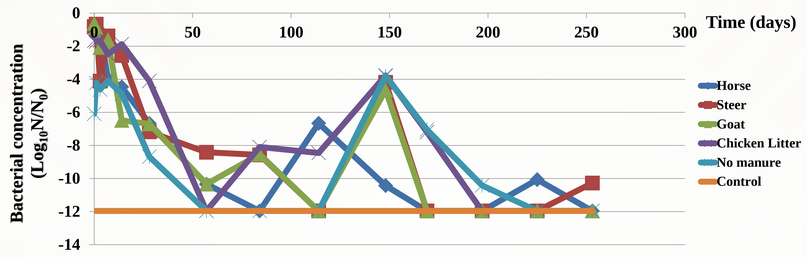

Figure 3: Survival of indicator *E. coli* inoculated in high (10^6-10^7 CFU/ml) bacterial concentrations in different manures spread on field plots

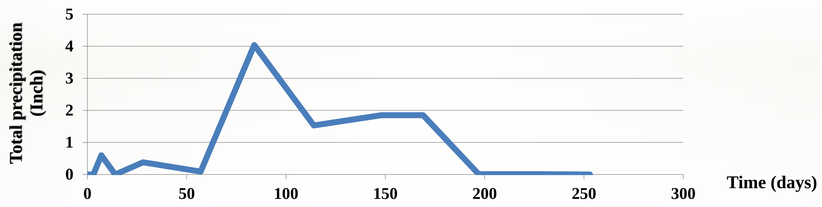

Figure 4: The total precipitation was measured in the Davis weather station #6, Sacramento Valley provided from the California Irrigation Management Information System (CIMIS)

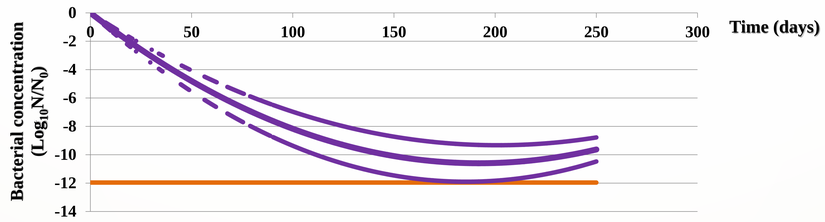

Figure 5: Predicted model for the survival of generic *E. coli* ($log_{10}N/N_0$) with 95% CI in soil amended with chicken litter (table 1) compared with control as a function of time, based on high bacterial concentration from the MPN method.

Table 1: The linear regression model with predictive mode was used to determine the amount of a cocktail indicator *E. coli* in manure amended soil ($log_{10}N/N_0$) using MPN determination method within 9 months

Predictors	Coefficients	95% C.I.	P-value
Constant	-3.943	-4.892 to -2.995	< 0.0001
Linear term (time)	-0.0786	-0.0939 to -0.0634	< 0.0001
Quadratic term (time^2)	0.000185	0.00012 to 0.000249	< 0.0001
Fecal type			
Horse manure	1.508	0.361 to 2.656	0.010
Cattle manure	1.376	0.228 to 2.522	0.019
Goat manure	0.967	-.0180 to 2.114	0.098
Chicken litter	1.884	0.737 to 3.031	0.001

AIC = 2902.602 1431.993

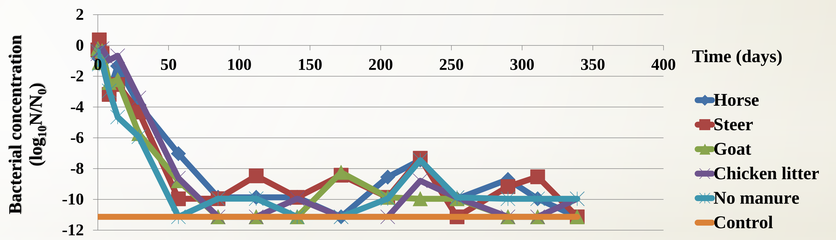

Figure 6: Survival of indicator *E. coli* inoculated in high (10^6-10^7 CFU/ml) bacterial concentrations in different manures spread on tomato field

- A significant log reduction in *E. coli* levels in amended soil was observed within 60 days from both high and low inoculum.
- All tomato samples (outside and inside) tested negative for the generic *E. coli* that was inoculated into soil amended with manure.
- The mean total bacterial count was positive at 1.41×10^6 CFU/tomato, with a range of 0 to 1.37×10^7 CFU/tomato. The mean Coliform bacteria detected was 2.46×10^6 CFU/tomato, with a range of 0 to 4.04×10^7 CFU/tomato.

Conclusions

Although die-off of *E. coli* was observed in soil 120 days after inoculation of manure amended soil with *E. coli* during a fall-winter period, resuscitation was observed for all manure types following heavy spring rains. The findings suggest that generic *E. coli* experiences multiple log reductions over 120 days, but exposures to rain fall can temporarily reverse these reductions. These results indicate that the use of raw or untreated manure may be a source of field contamination with pathogens. However, no *E. coli* contamination of tomatoes from the manure amended soils was observed in this limited study. More studies are needed to characterize the long term effects of rain, and the probability of transfer of pathogens from manure amended soils to tomatoes.

Acknowledgements

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