Iowa State University

USDA-ARS

Effect of Swine Manure Application Timing on the Persistence and Transport of Antibiotic Resistant Enterococcus and Resistance Genes in Tile-drained Fields

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BACKGROUND INFORMATION

- Fields receiving swine manure have the potential to transport antibiotic resistant pathogenic bacteria and their associated resistance genes outside of agricultural settings.
- Iowa livestock produce 10 billion gallons of manure annually.
- Different manure application timing, tillage, and crop rotations may impact the persistence and transport of antibiotic resistant bacteria and antibiotic resistance genes.
- The longer resistant bacteria and resistance genes persist in agricultural soils, the longer they are at risk to be transported from the system through tile drainage.

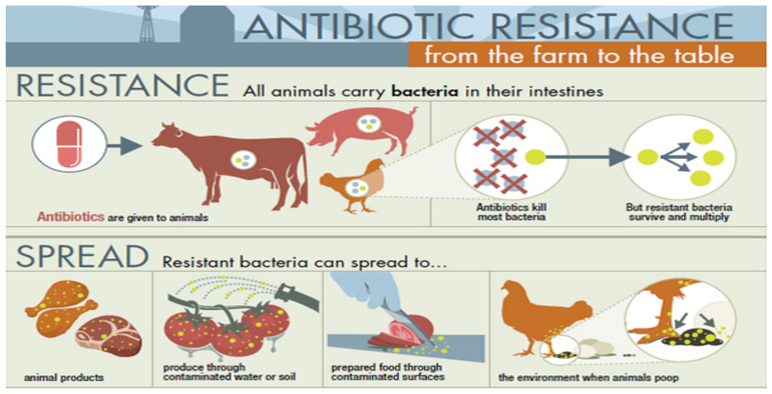
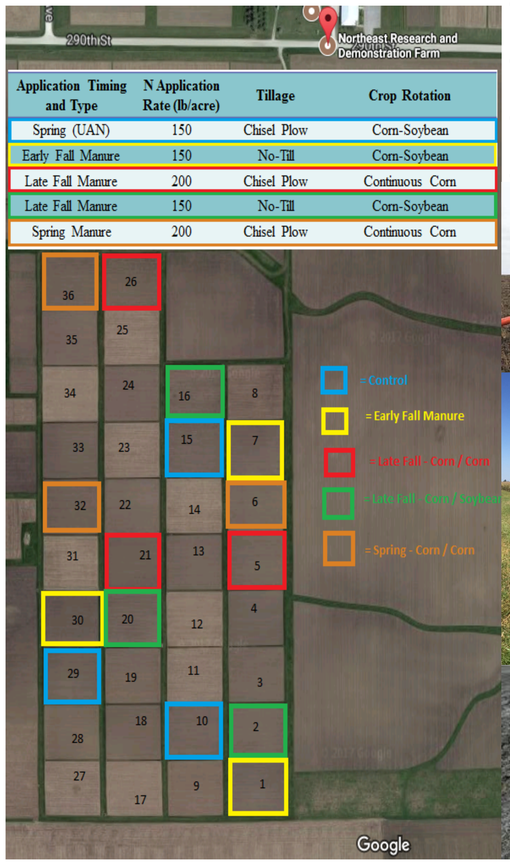


Image taken from http://www.mrsaidblog.com/2016/12/22/from-farm-to-fork-how-does-giving-antibiotics-to-livestock-result-in-superbugs-on-your-plate

RESEARCH OBJECTIVES

- Compare the persistence of Enterococcus and antibiotic resistant Enterococcus.
- Determine the effect of application timing and crop rotation on the persistence of Enterococcus and *ermB*.
- Determine if *ermB*, which results in resistance to tylosin, decays at the same rate as tylosin-resistant Enterococcus.

MANURE APPLICATIONS + SAMPLING

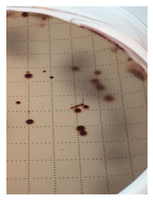


Application Timing and Type	N Application Rate (lb/acre)	Tillage	Crop Rotation
Spring (UAN)	150	Chisel Plow	Corn-Soybean
Early Fall Manure	150	No-Till	Corn-Soybean
Late Fall Manure	200	Chisel Plow	Continuous Corn
Late Fall Manure	150	No-Till	Corn-Soybean
Spring Manure	200	Chisel Plow	Continuous Corn

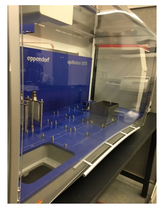
- Four manure treatments + UAN control
- 15 cm soil cores in and between manure bands
- Analyze for Enterococcus and Resistance Genes
- Sample 5-7 time points over 6-month span

PROCESSING + ANALYSIS

Sieve, Subsample

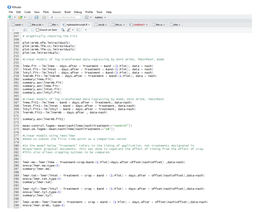


Membrane Filtration

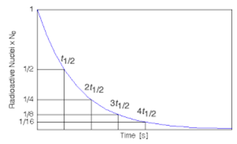


DNA Extraction

qPCR



R – Data Processing + Analysis



R – Half-Life Determination

RESULTS + STATISTICS

Half Lives of Analytes (Days)

Log-Response Variables	Early Fall Manure Half-Life	Early Fall Manure Standard Error	Late Fall Manure Half-Life	Late Fall Manure Standard Error	Spring Manure Half-Life	Spring Manure Standard Error
Enterococcus	50.410	0.004	23.75	0.006	8.11	0.015
Tetracycline Res. Enterococcus	51.03	0.005	24.53	0.006	6.70	0.012
Tylosin Res. Enterococcus	60.53	0.005	23.17	0.005	6.85	0.019
ermB	82.06	0.004	31.58	0.007	15.12	0.016

Contrasts (T-Tests - α<.05)

Log-Response Variables	Early Fall Manure t	Early Fall Manure p-value	Late Fall Manure t	Late Fall Manure p-value	Spring Manure t	Spring Manure p-value
Ent. vs.Tetracycline Ent.	0.316	0.754	17.434	0.000	6.064	0.000
Ent. Vs. Tylosin Ent.	2.708	0.009	1.247	0.221	-3.509	0.002
Tylosin Ent. Vs. *ermB*	3.765	0.001	0.000	0.005	-17.409	0.000
Ent. Vs. *ermB*	-57.022	0.000	4.741	0.000	30.492	0.000

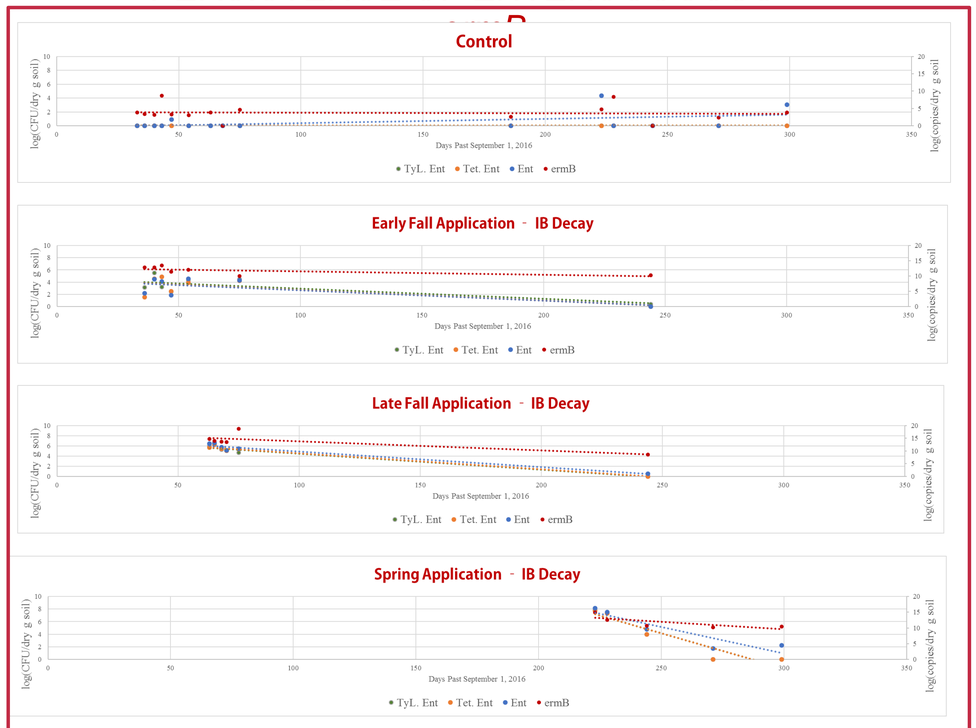
Linear Mixed Effect Regression Model:

Response	Treatment	Crop	Band	Days After
log(Enterococcous)	0.006	0.178	2.20E-16	3.11E-07
log(Tetracycline Res. Enterococcus)	6.36E-05	0.046	2.20E-16	7.98E-10
log(Tylosin Res. Enterococcus)	1.49E-05	0.002	2.20E-16	7.10E-09
log(*ermB*)	1.17E-10	0.185	2.20E-16	0.156

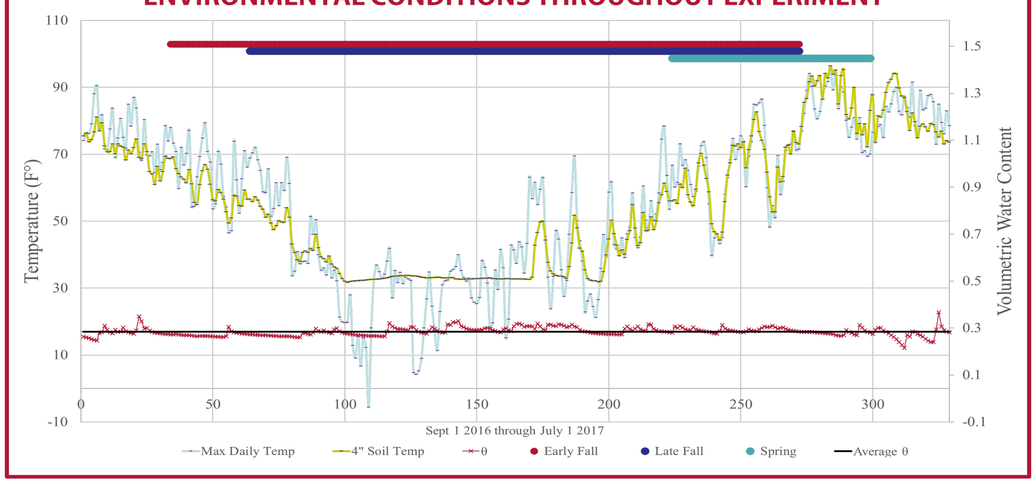
p < .001
p < .01
P < .05
p < .1
p > .1

- This statistical model was chosen to understand the effects of manure application timing and crop rotation on the persistence of Enterococcus and ermB.
- Factor variables: treatment, crop, band
- Continuous Variables: days after application
- Random Variables: plot

PERSISTENCE OF ENTEROCOCCUS + [illegible]



ENVIRONMENTAL CONDITIONS THROUGHOUT EXPERIMENT



CONCLUSIONS

- Spring application resulted in the shortest half-life for all analytes. Early Fall resulted in the longest. This could be explained by lower temperatures in the fall, changes in the soil biota
- Application timing has a significant effect on the persistence of Enterococcus, Enterococcus resistant to tetracycline, and Enterococcus resistant to tylosin.
- Crop rotation did not have a significant effect on differences in the persistence of Enterococcus or *ermB*.

FUTURE RESEARCH

- Determine the persistence of another macrolide resistance gene, and three tetracycline resistance genes.
- Compare the persistence of resistance genes conferring the same type of resistance (e.g. macrolide, tetracycline).
- Compare the persistence of antibiotic resistance genes with different mechanisms of resistance (e.g. efflux pump, ribosomal protection).

ACKNOWLEDGEMENTS

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