A Presentation of the

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November 23, 2010 Owatonna, MN

11th Annual Drainage Research Forum

Owatonna, Minnesota

November 23rd, 2010

Transport of antibiotic resistant bacteria into tile drainage systems

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Background/Motivation

Increasing antibiotic resistance

Antibiotic resistance on the rise

By Anita Manning, USA TODAY

In the battle between bugs and drugs, the bugs are scoring some big wins.

http://www.usatoday.com/news/health/2002-09-29-drugs-usat_x.htm

Antibiotic Resistance Growing as US Health Risk

http://www.aolnews.com/health/article/antibiotic-resistance-growing-as-us-health-risk/19454589

Drug-resistant strain of E. coli emerges in U.S.

New strain may be on way to becoming "untreatable," researchers say

http://www.msnbc.msn.com/id/38495130/ns/health-infectious_diseases/

Antibiotic-Resistance DNA Showing Up in Drinking Water

http://www.foxnews.com/story/0,2933,227106,00.html

http://blog.healia.com/00365/new-swine-flu-outbreak-us-and-mexico-start-flu-pandemic

Image by Christina Goeddel

Pathogens are a leading cause of water quality impairments in streams and rivers

http://greenerloudoun.wordpress.com/2009/02/16/thanks-for-the-free-cow-urine-in-my-drinking-water/

- Agricultural practices
  - Leading source of all impairments
- Causes of waterborne disease outbreaks resulting in gastroenteritis
- Enterococci are a potential indicator of pathogens
- Developing resistance to many antibiotics

Microbial Indicator	Federal Standard for primary contact
Fecal coliform	200 CFU/100 mL
E. coli	126 CFU/100 mL
Enterococci	33 CFU/100 mL

Factors we considered in this study:

1. Representative scenario

- 1/3 of land area in Iowa is drained
- Bacteria move into tiles via macropores
  - Consider only no-till
- Tylosin commonly administered at sub-therapeutic levels
  - Up to 90% excreted

2. What is lacking?

- Relationships between sources and resistant organisms typically inferred

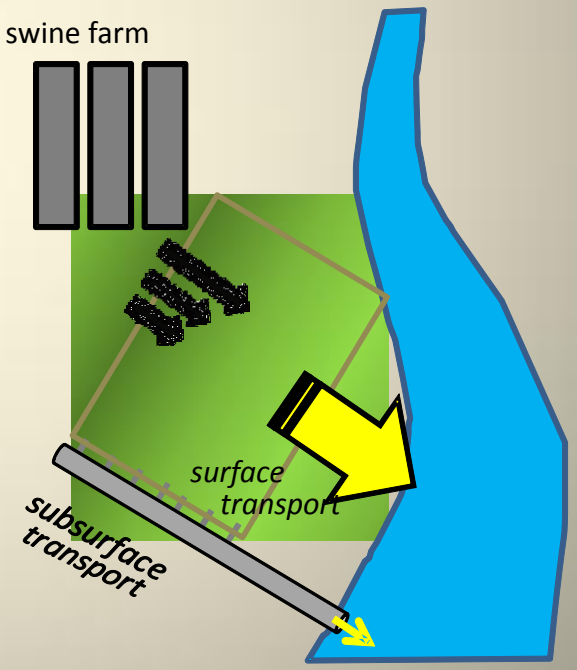
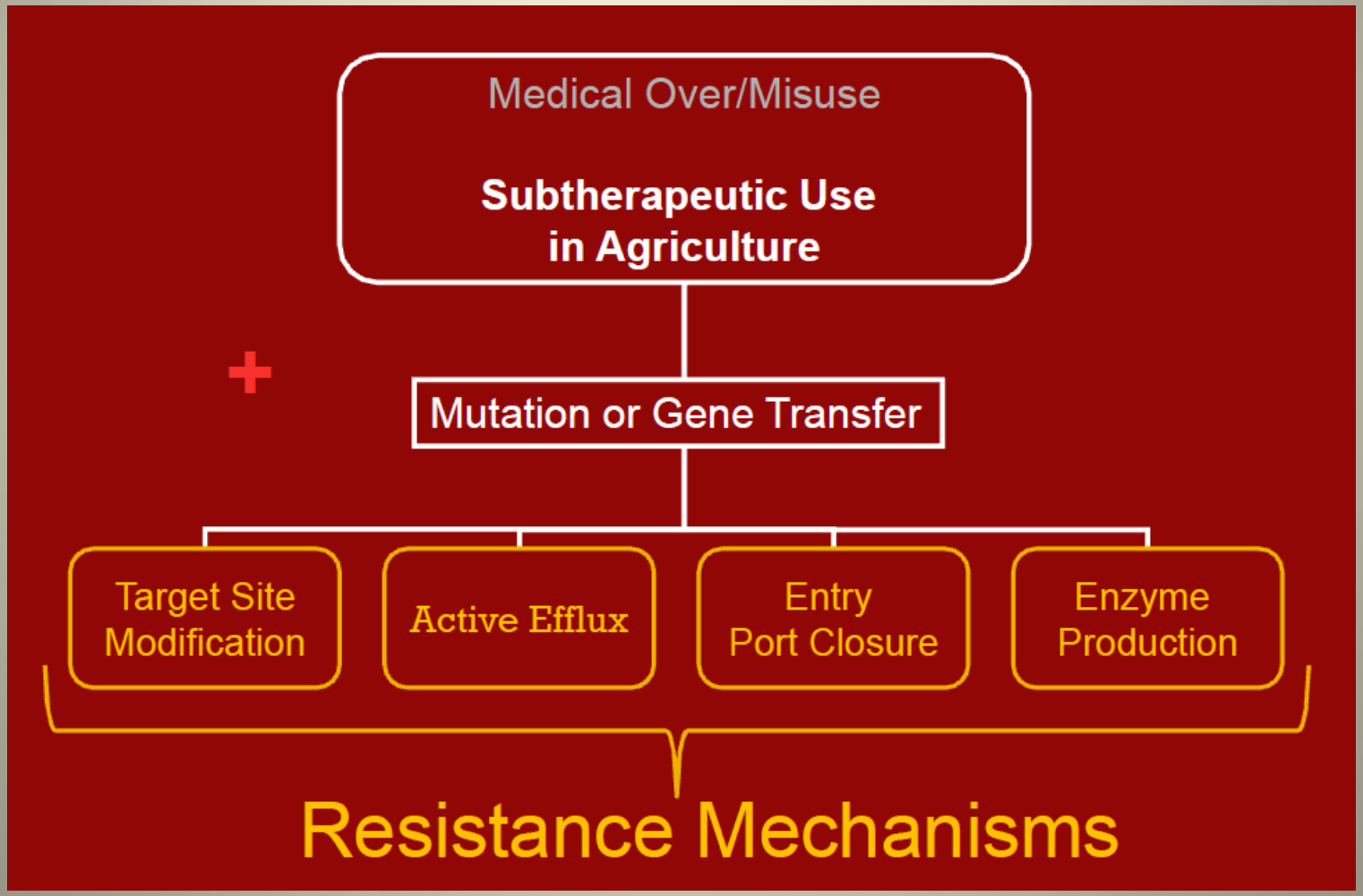


Image by Alok Bhandari

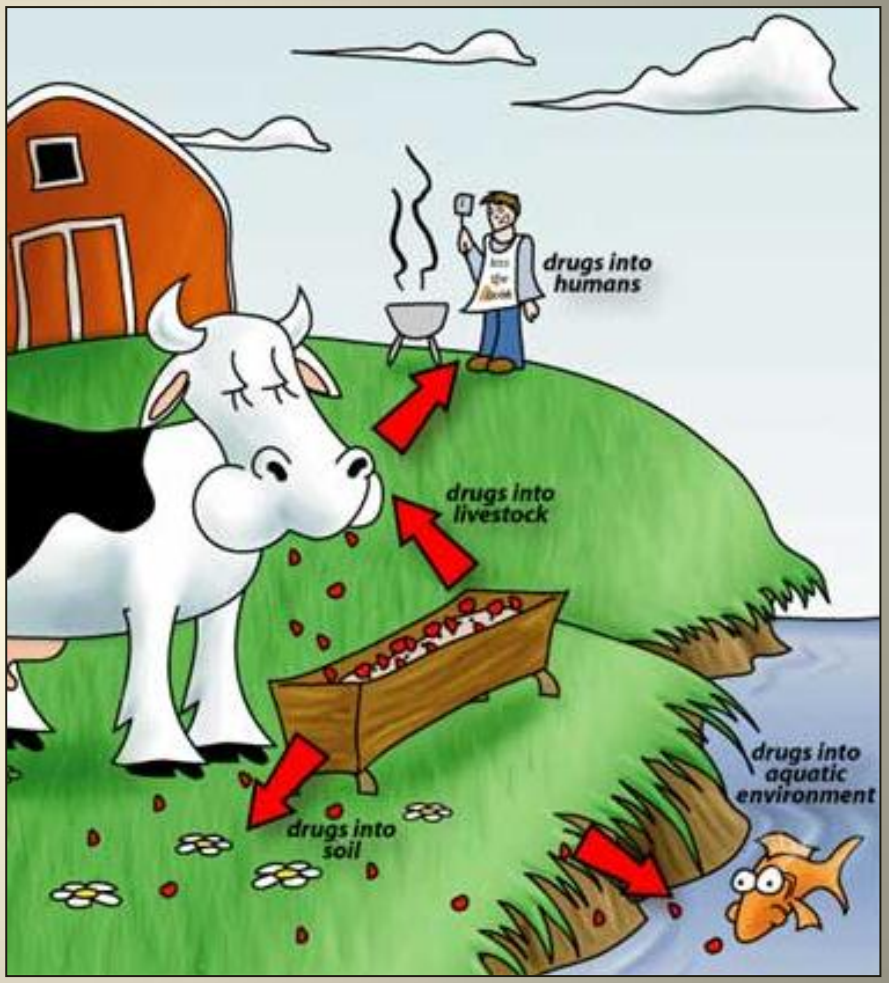
Factors we considered in this study:

3. **What is causing the resistance?**



Objectives of the study:

1. To detect and quantify the occurrence of tylosin-resistant enterococci in:
   - manure from swine facilities feeding tylosin at sub-therapeutic doses;
   - soils amended with swine waste and
   - tile drain flow from swine waste amended agricultural fields;
2. To assess the effects of hydrology and solids transport on the enterococci in tile water;
3. To test enterococci isolates for known macrolide resistant genes

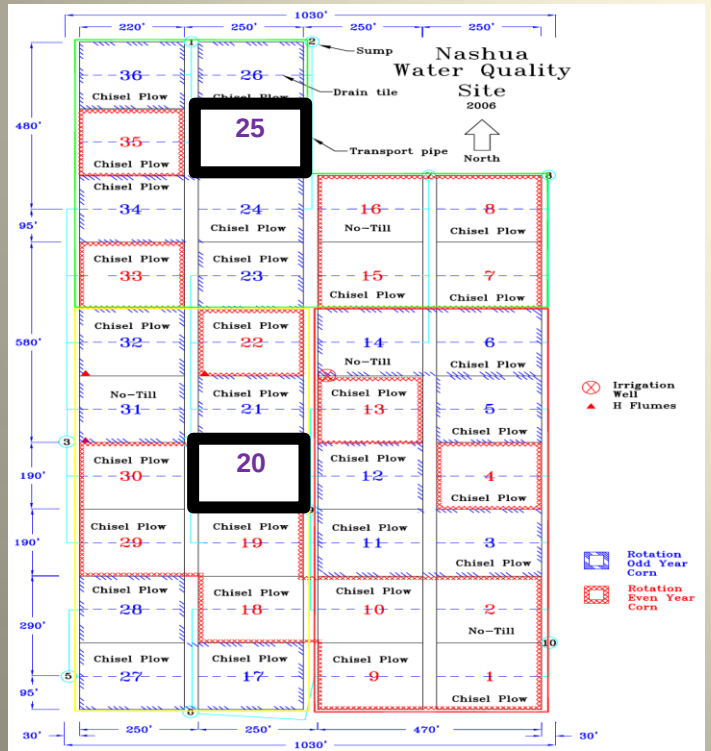


www.scq.ubc.ca

Approach

Field Study: Nashua, Iowa

- ISU Northeast Research Farm
- 36 plots (0.4 ha each)
  - Soil type: moderate to poorly drained
  - Tile drainage: 1979
- Corn-soybean rotation



Manure & Soil Sampling

Approach

Rainfall Simulation

- ISU Northeast Research Farm
- 36 plots (0.4 ha each)
  - Soil type: moderate to poorly drained
  - Tile drainage: 1979
- Corn-soybean rotation

Water Sampling

- Spring: sampling started after flow started
- Fall: sampling started after rainfall simulation started

Approach

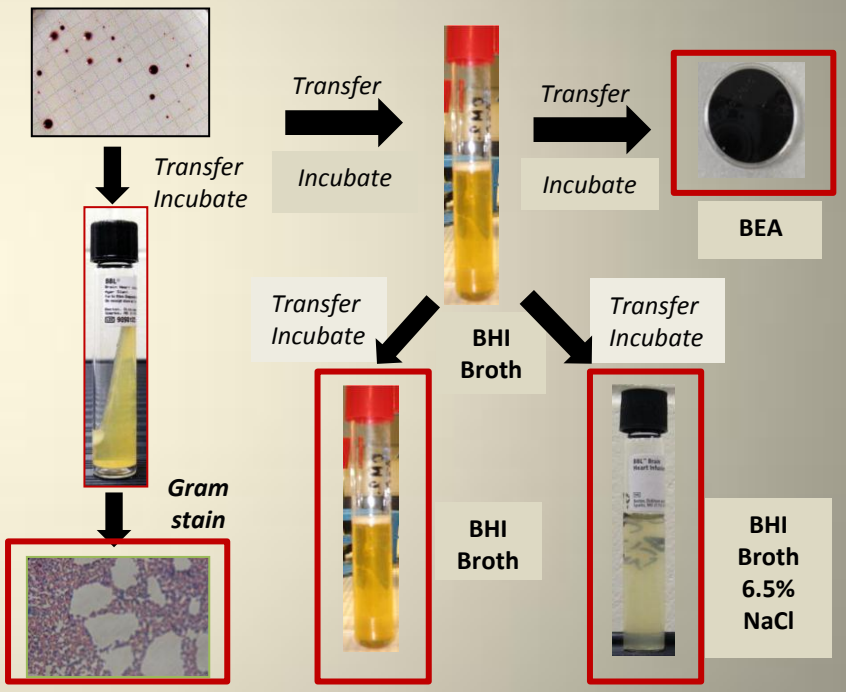
Analysis for Enterococci

- Membrane Filtration Technique (APHA, 1998)
- mEnterococcus agar with tylosin at 35 mg/L (resistant) and without tylosin (control)

- Total Suspended Solids

Confirmation

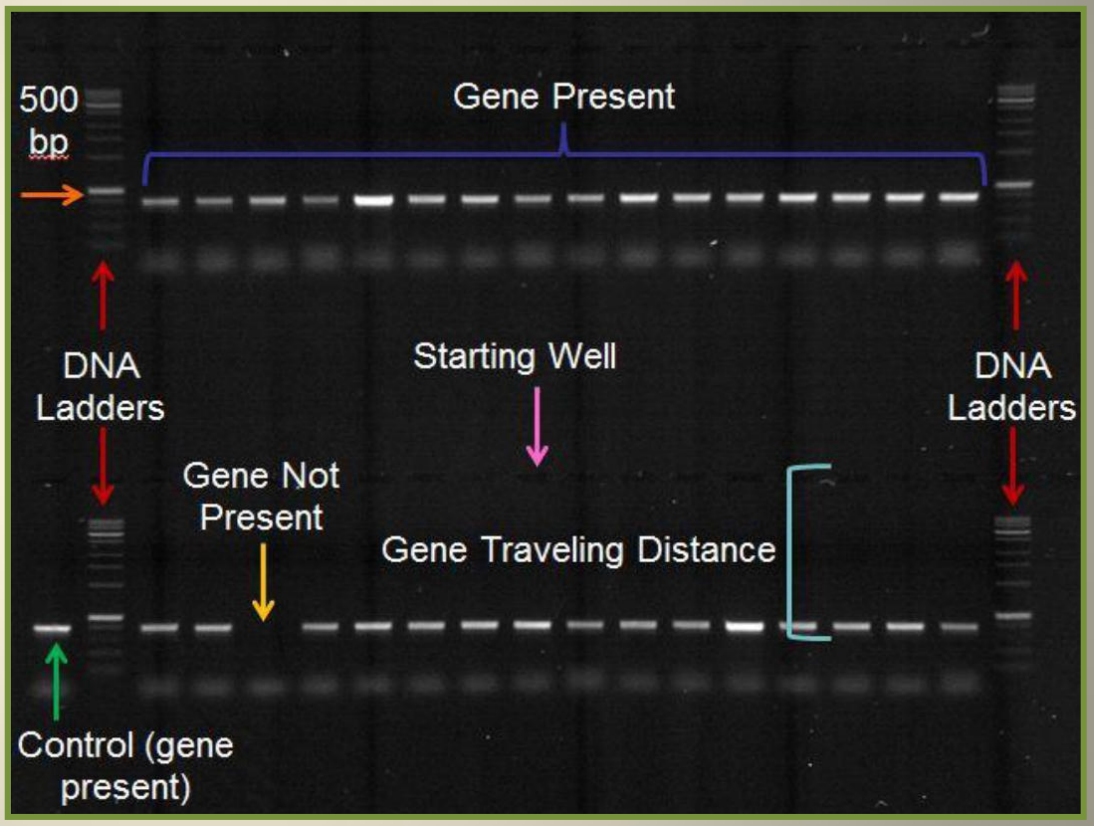
- EPA Method 1600



Approach

Resistant Gene Detection by PCR

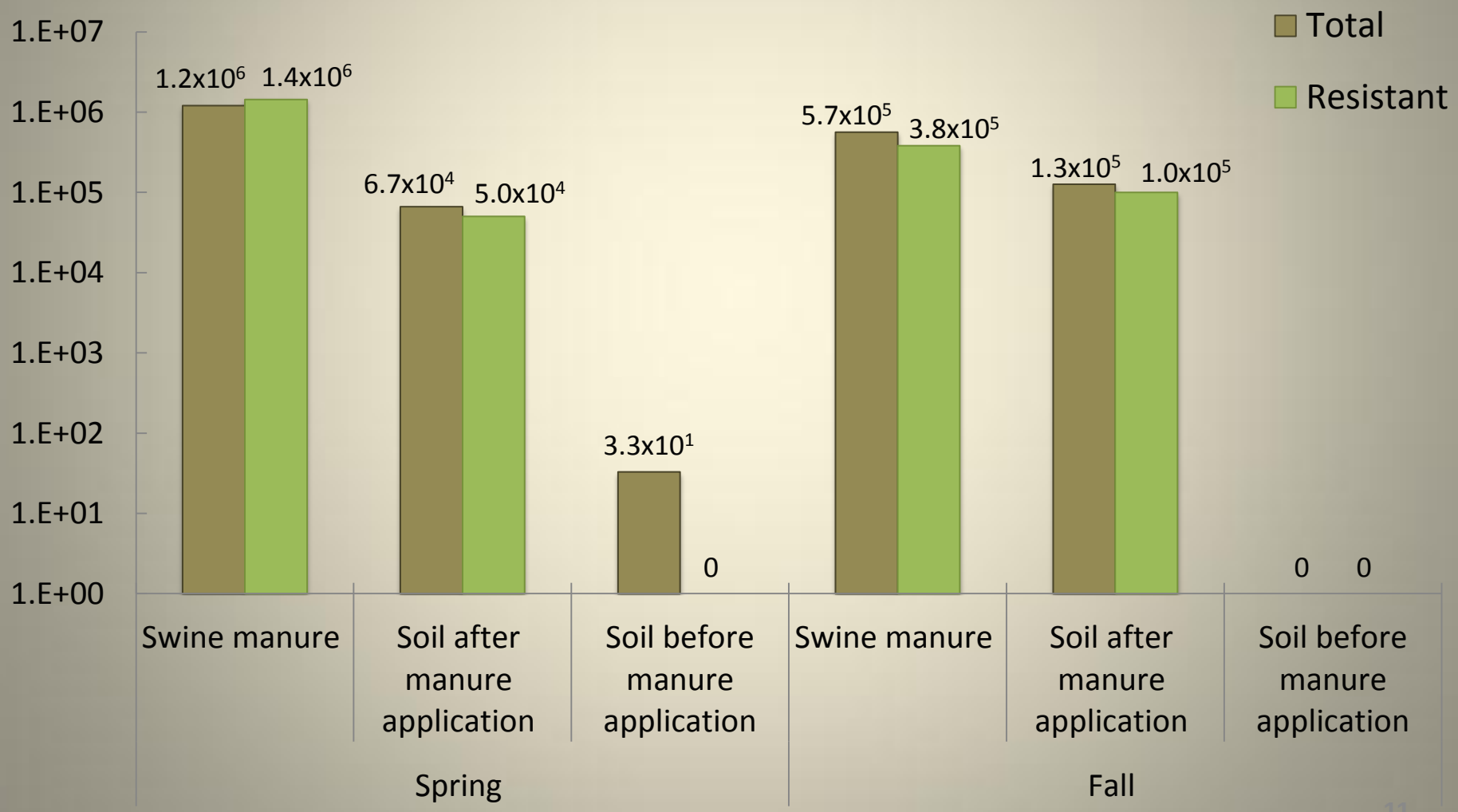
- Select isolates
- DNA extraction
- PCR Amplification (Denaturation, annealing, and extension)
- Positive controls: Dr. Roberts, University of Washington
- Negative controls: ATCC



Mechanisms of Resistance	Genes with Mechanism
Active Efflux	mef(A), msr(A)
Target Site Modification	erm(A), erm(B), erm(C), erm(F), erm(T), erm(X)

Results

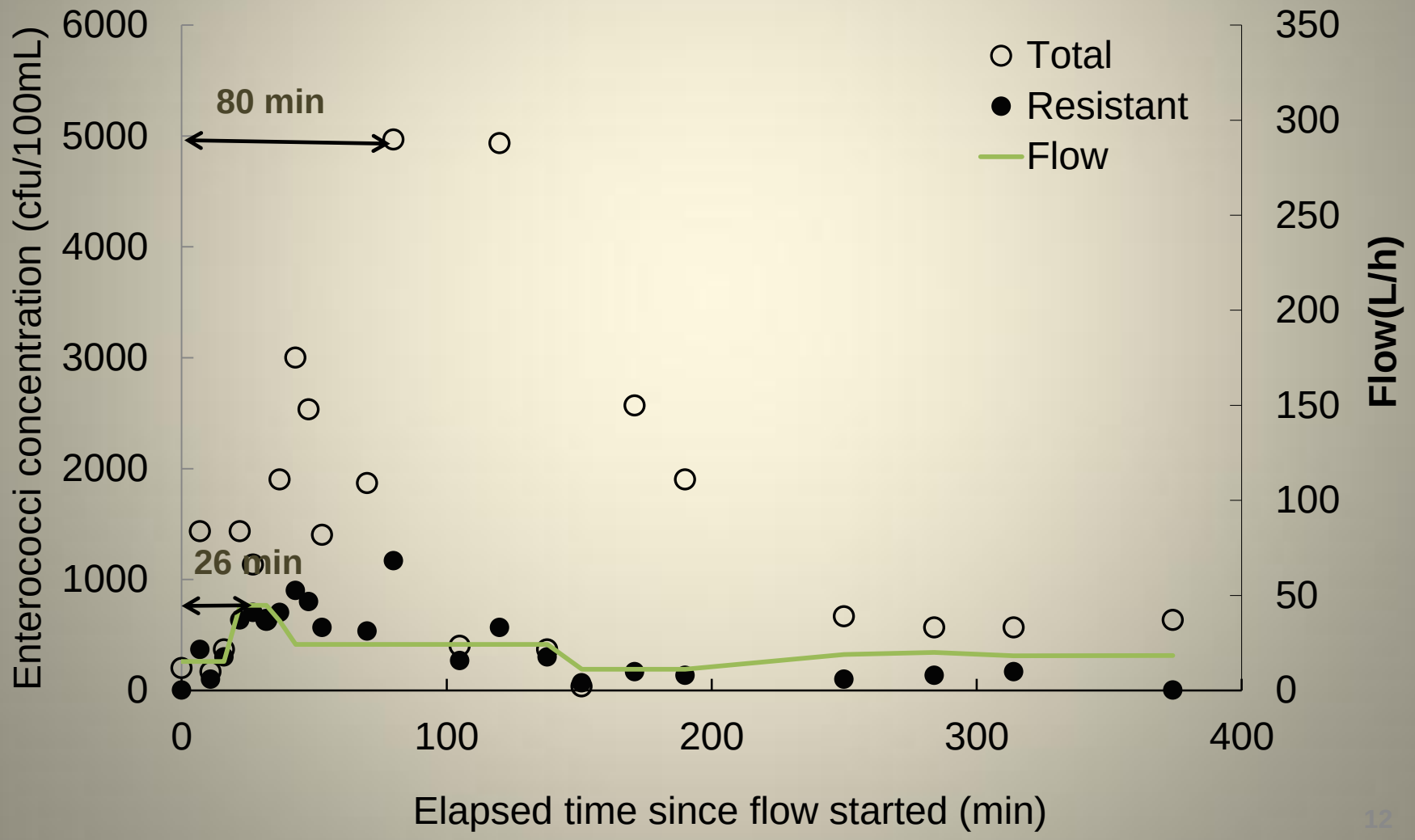
Enterococci in manure and soil samples



Enterococci and tile flow

Spring 2009

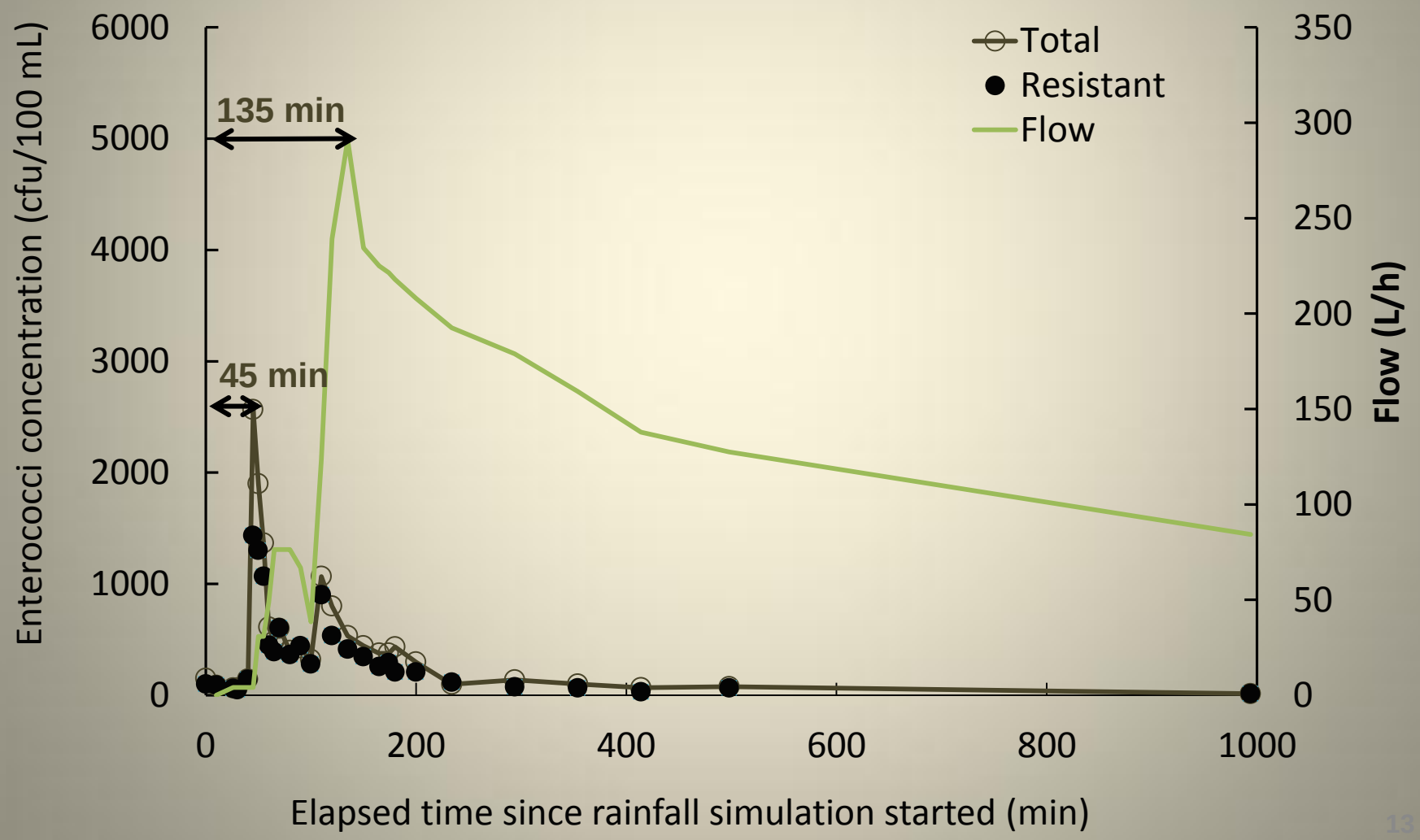
Total : 33 to 4967 CFU/100 mL
Resistant: 67 to 1167 CFU/100 mL



Enterococci concentration and tile drained flow

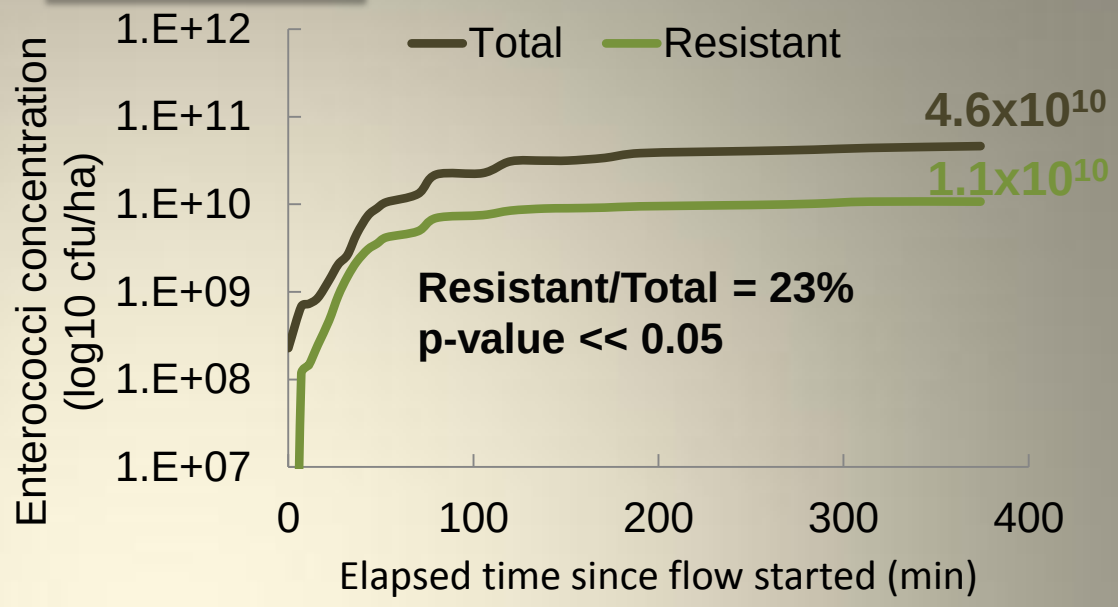
Fall 2009

Total : up to 2567 cfu/100 ml
Resistant: up to 1433 cfu/100 ml

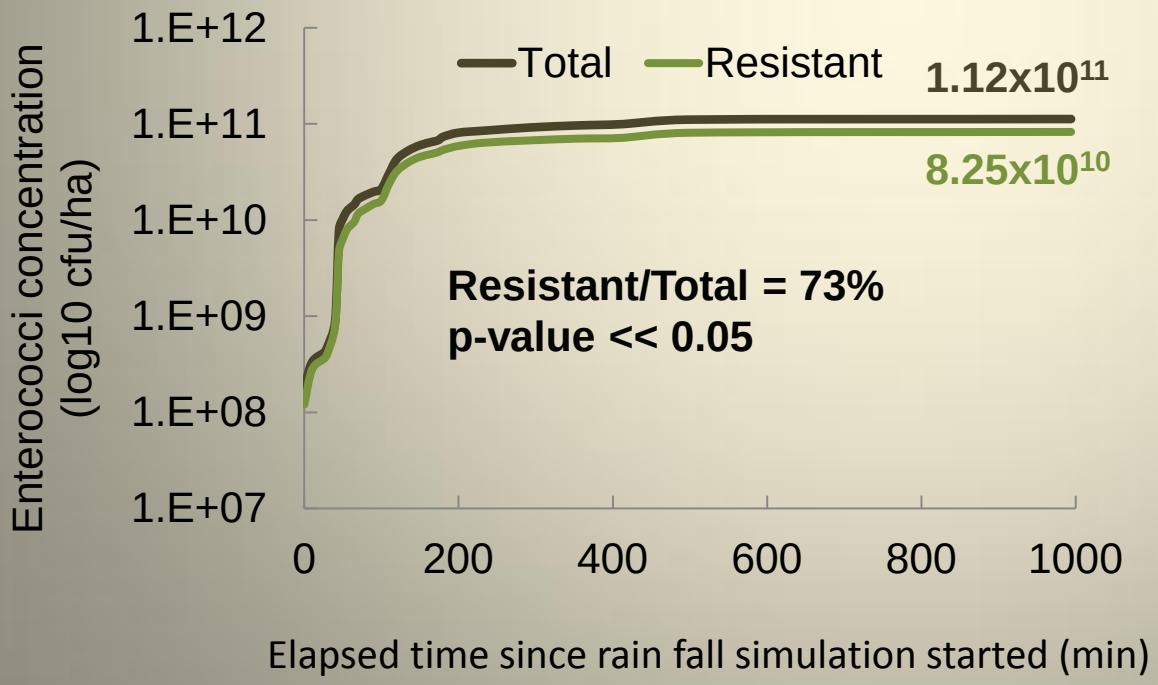


Results: Enterococci loading cfu/ha

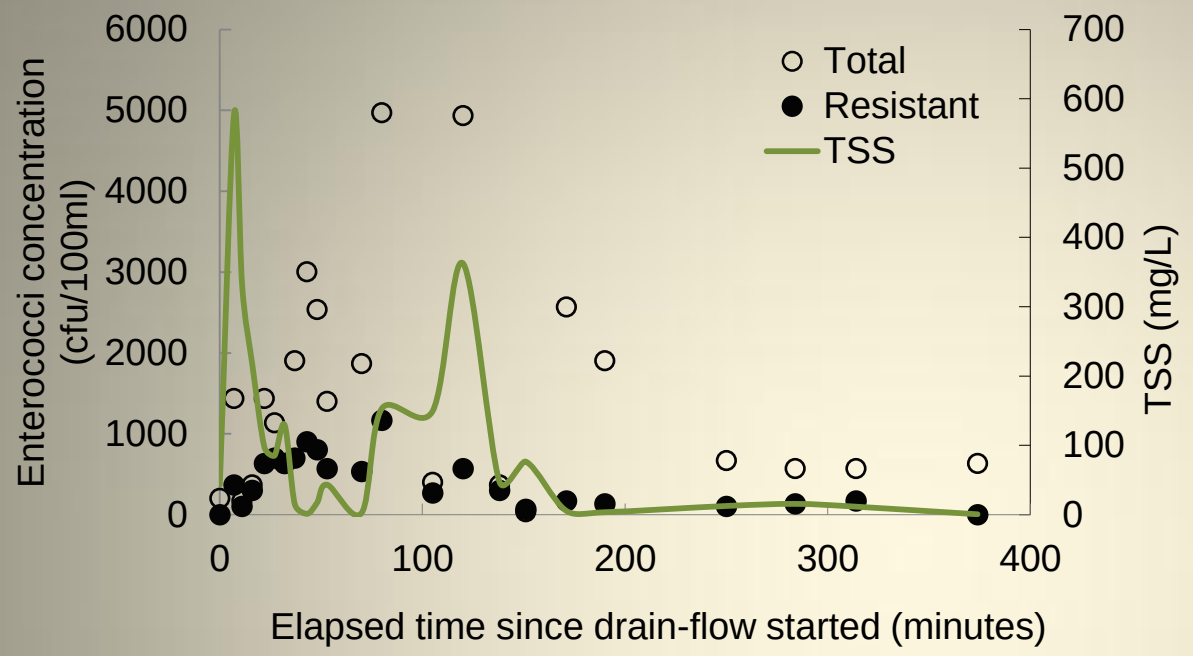
Spring 2009



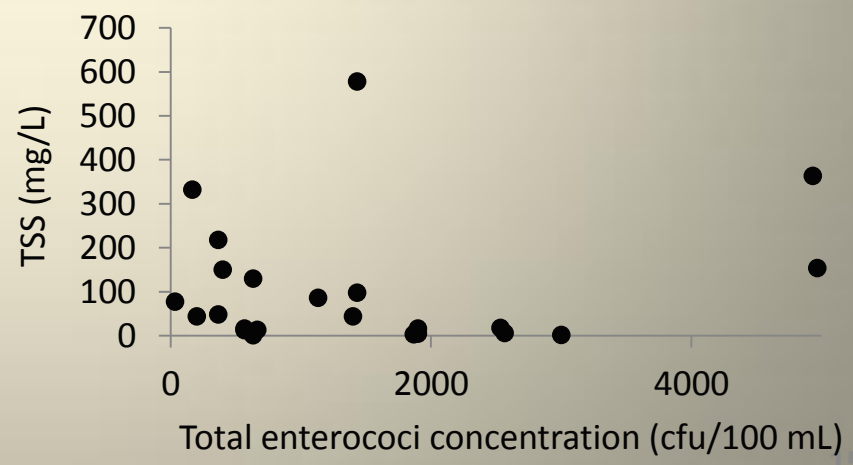
Fall 2009



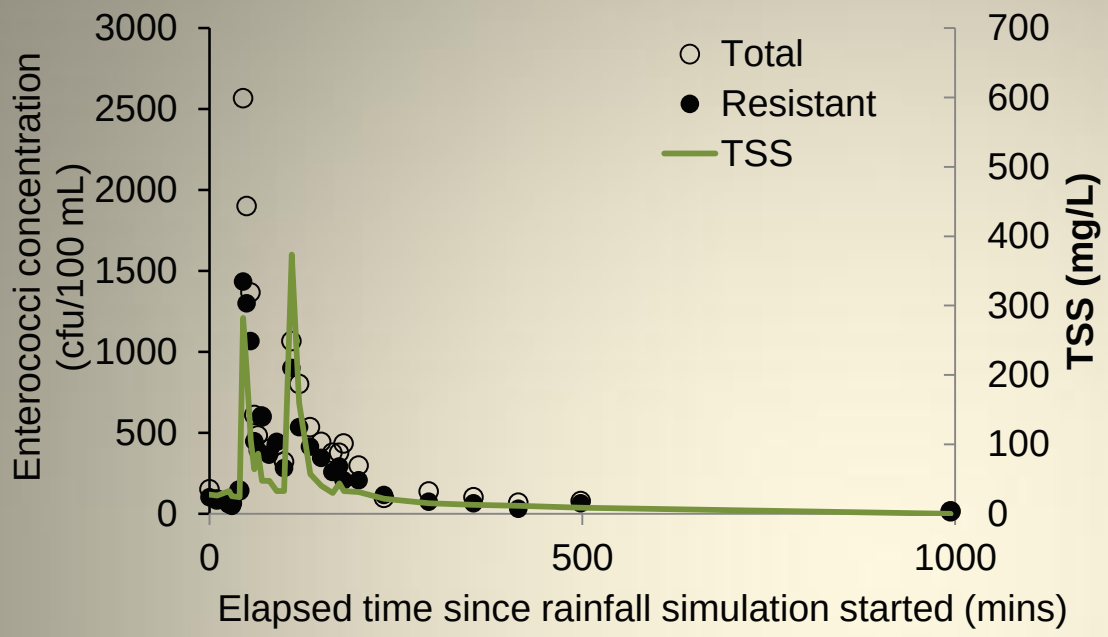
Enterococci concentration and TSS: Spring Simulation

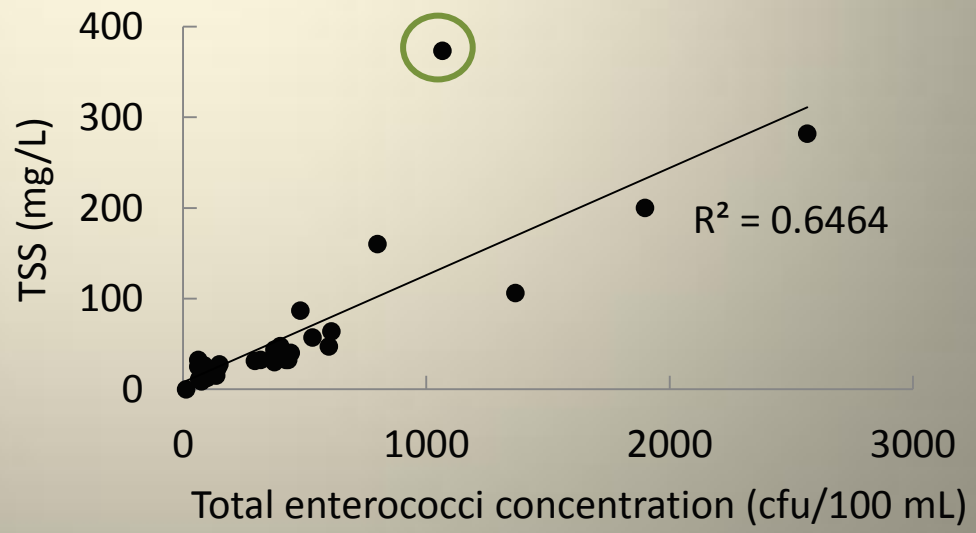


- Poor regression because sediment was deposited in the tile line



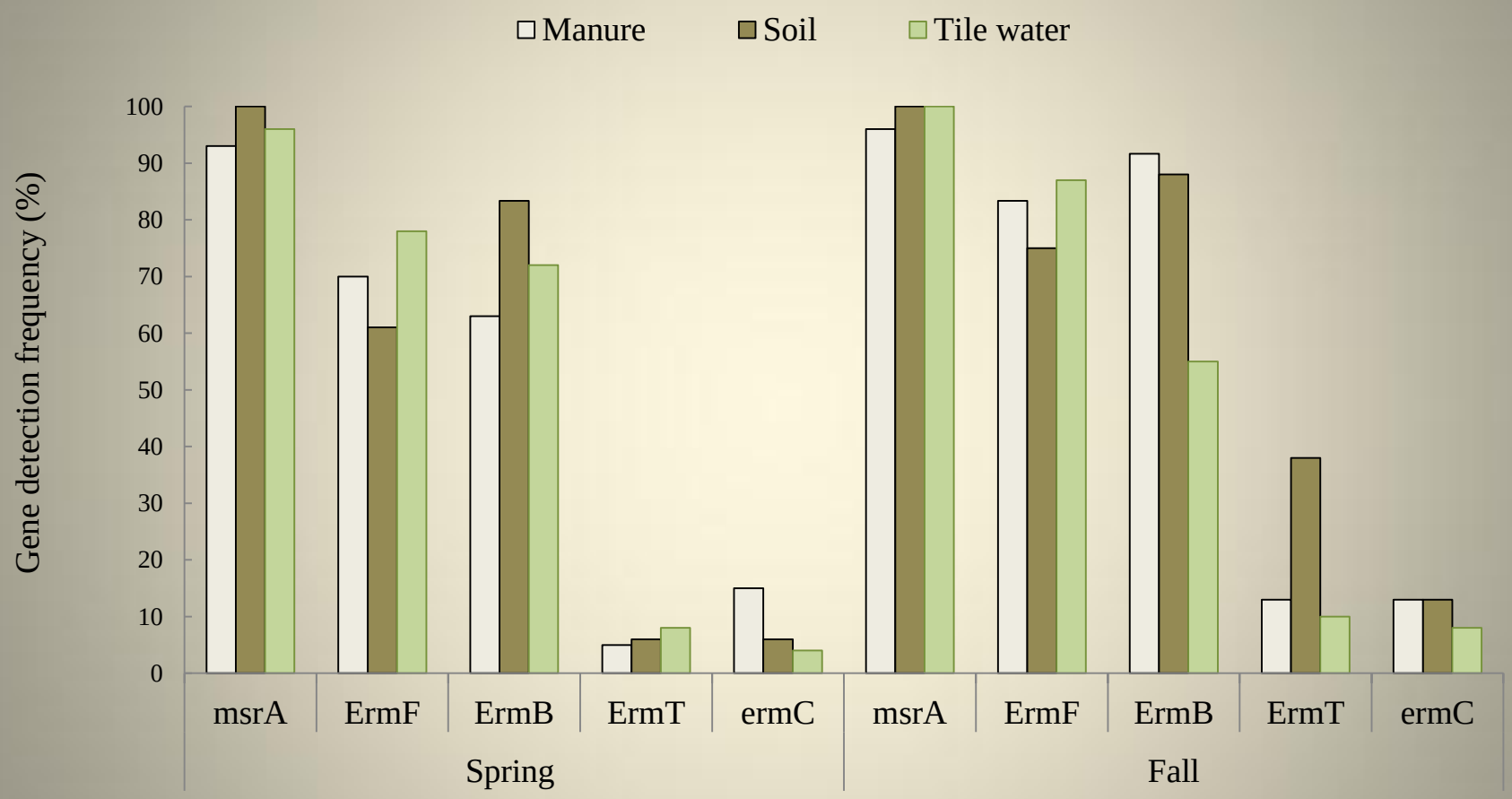
Enterococci concentration and TSS: Fall Simulation





- Good correlation because of base -flow

Resistant gene detection



Conclusions

1. This study has provided the first data on tracking the transport pathway of tylosin-resistant enterococci from a specified source through a subsurface drainage system
   - Transport of resistant enterococci from swine manure through soil and into tile drainage was confirmed
   - Enterococci control and tylosin –resistant concentrations were significantly different

2. In the presence of base-flow, good correlation between TSS and enterococci was observed.

3. Tylosin resistant genes were detected in enterococci isolates from manure, soil and tile water: msrA, ermB, ermF, ermT, ermA, ermX.
   - Both resistance mechanisms to tylosin occurred in enterococci isolates.

Acknowledgements

- Multi-university USDA-NRI grant on Role of Directly Connected Macropores in Pathogen Transport to Subsurface Drainage
- VOSP scholarship from Vietnamese government
- Graduate & undergraduate students: Chi Hoang, Pramod Pandey, Martha Zwonitzer, Ping Liu, Rachel McDaniel, Christina Goeddel and Bridgette Huss