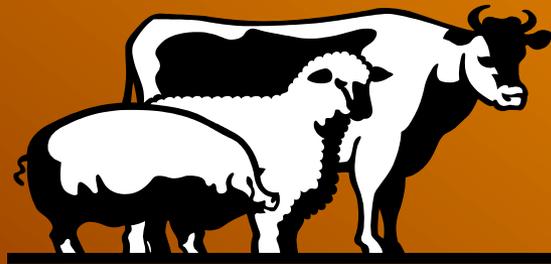




Prevalence of pathogens and antibiotic resistant bacteria in manure amended soil and feedlot runoff



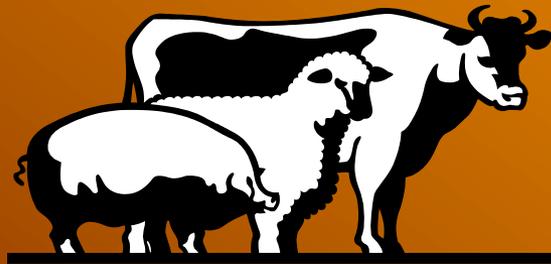
Rationale



- Maximize use of manure as a as nutrient source
- Minimize adverse environmental impacts
 - ✓ Pathogens
 - ✓ Antibiotic resistant bacteria



Runoff from manure amended fields





Rainfall simulation studies designed by John Gilley, Ph.D.

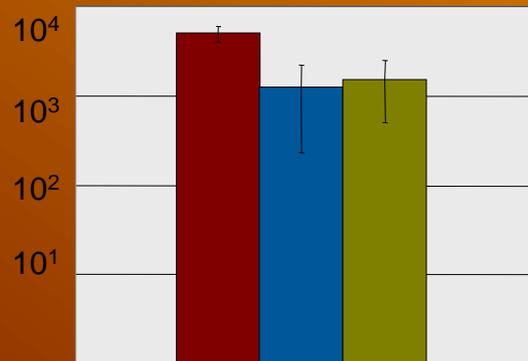




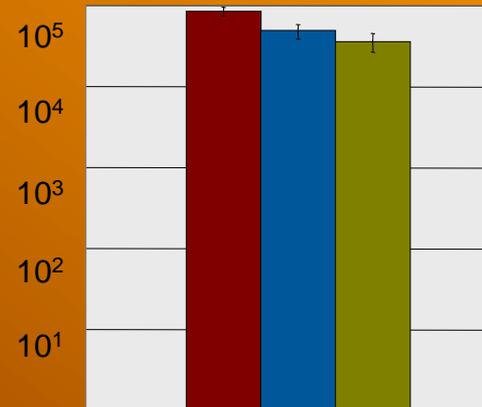
Results - pathogens and fecal indicator organisms

■ Manure ■ Runoff - no wheat strip ■ Runoff - wheat strip

Enterococcus



E. coli



Total Coliforms

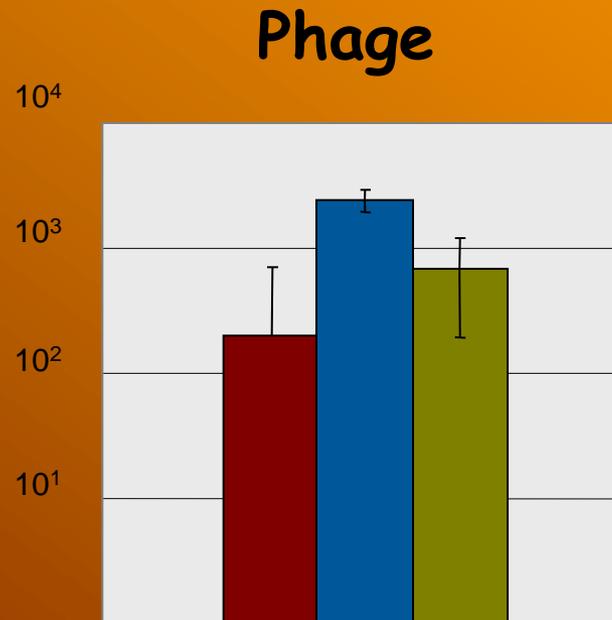


Summary of three rainfall simulations per plot for wheat and no residue, and all three manure application rates

Wheat strip is not effective at reducing transport of enteric bacteria in runoff from manure amended fields

Results - pathogens and fecal indicator organisms

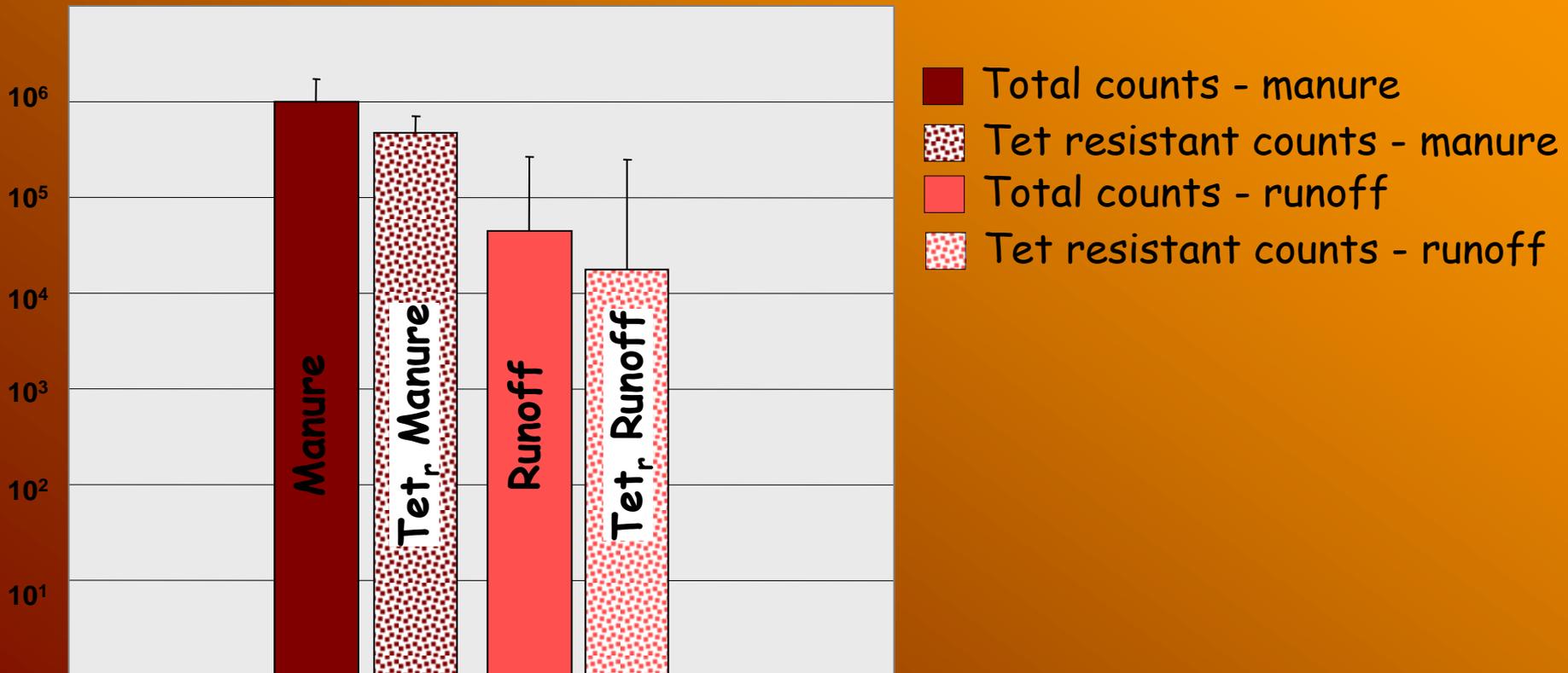
■ Manure ■ Runoff - no wheat strip ■ Runoff - wheat strip



Enumeration of Tetracycline Resistant Enteric Bacteria

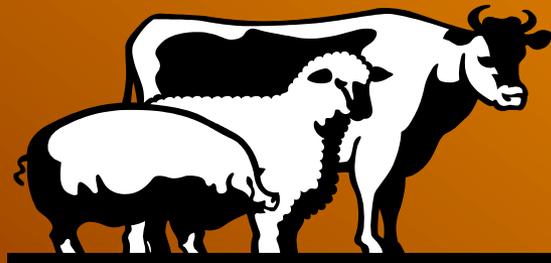
In simulated runoff from manure amended fields

Bacterial counts (cfu/ml) on MacConkey agar, and MacConkey with 8 ug/ml tetracycline





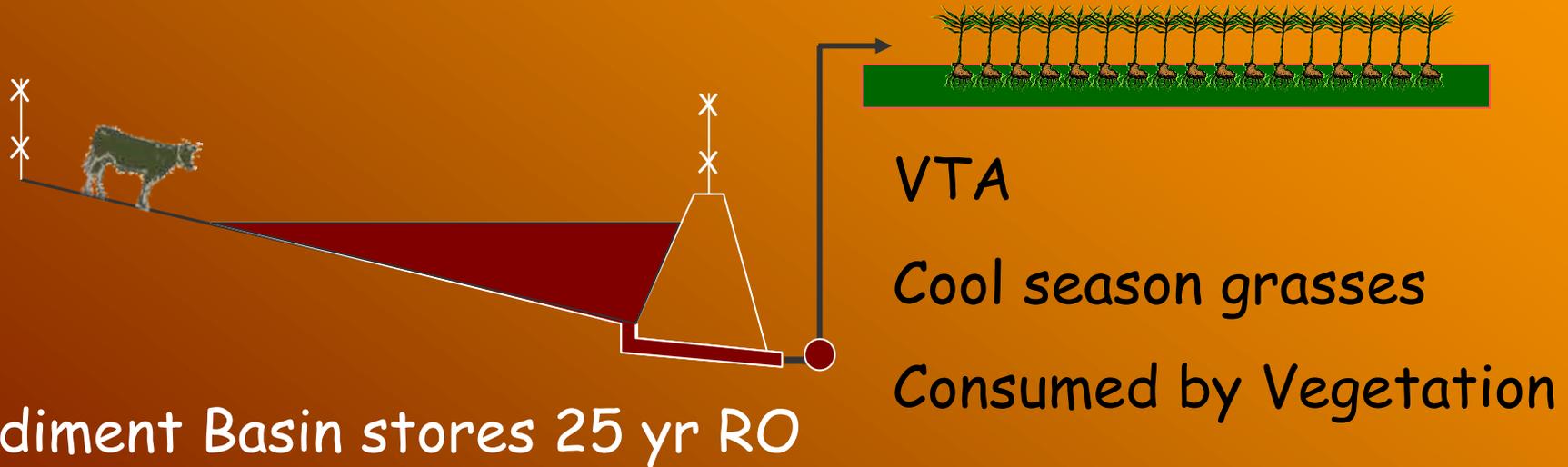
Feedlot Runoff - VTS



Nebraska VTS Design

Chris Henry, Ph.D

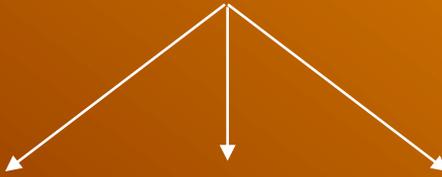
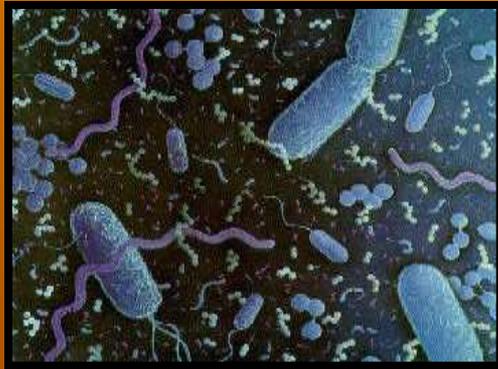
Traditional VTS relies on overland flow.
Dr. Henry's systems use pump.



Results - pathogens and fecal indicator organisms

	Quantify	Isolate
STEC O157	X	X
STEC O111		X
STEC O26		X
Salmonella		X
E. coli	X	
Phage	X	
Total Coliforms	X	
Clostridia		X
Enterococcus	X	

Relationships between antibiotic resistant bacteria and antibiotic resistant genes



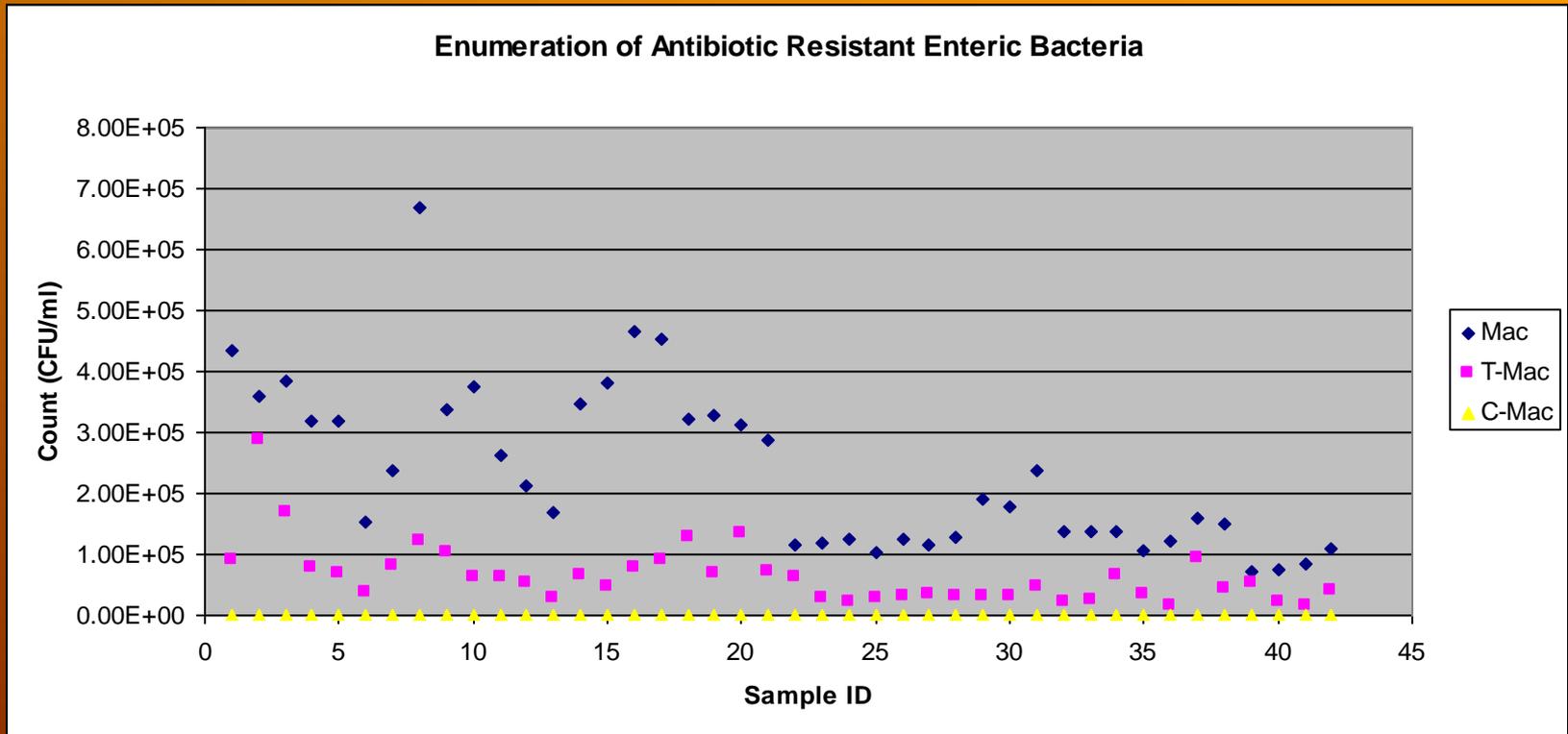
Phenotype



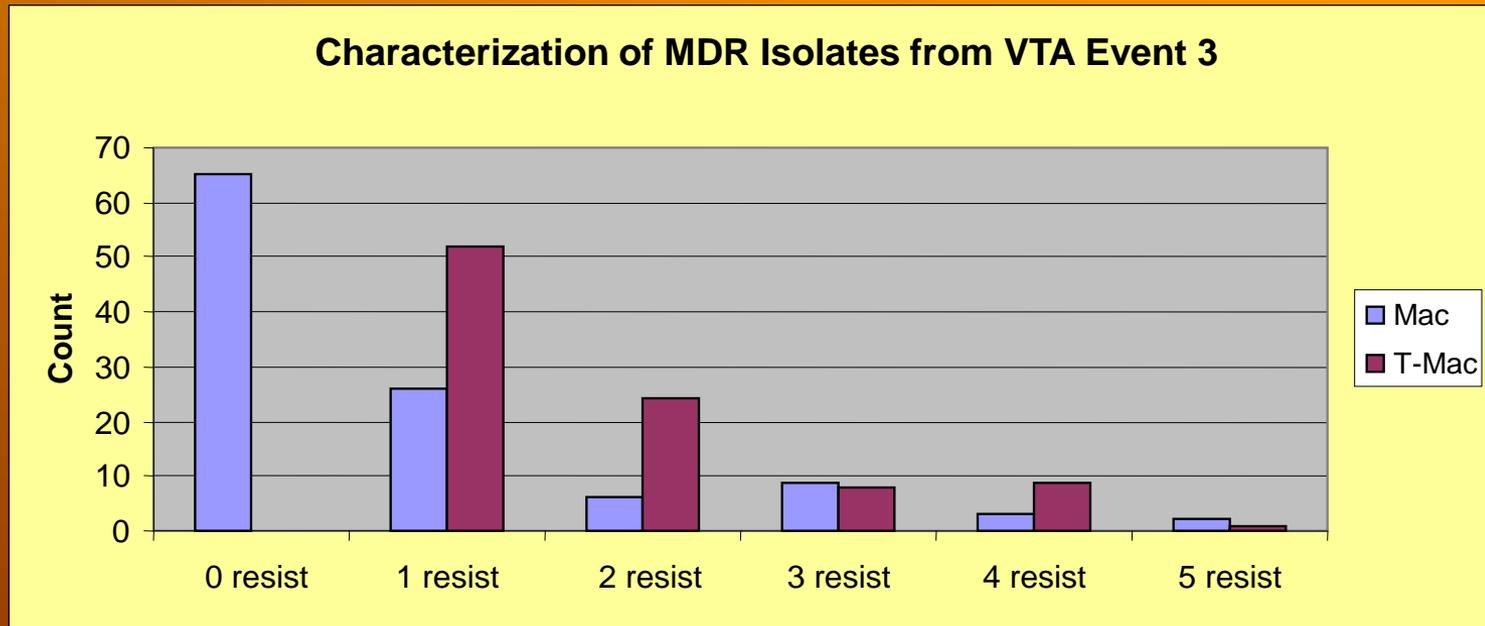
tet(B)	tet(C)	tet(D)
tet(A)	tet(E)	tet(G)
tet(K)	tet(L)	tet(M)
tet(O)	tet(S)	tet(P)
tet(Q)	tet(X)	

Genotype

Enumeration of antibiotic resistance in enteric bacteria



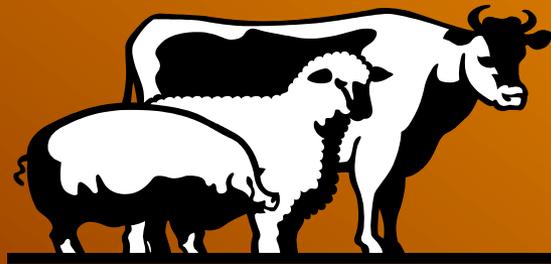
Multi-drug resistance in enteric bacteria

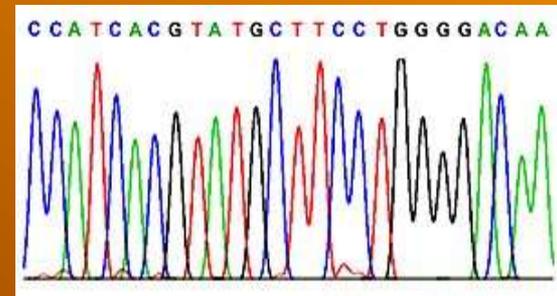
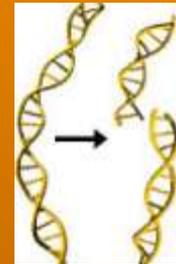
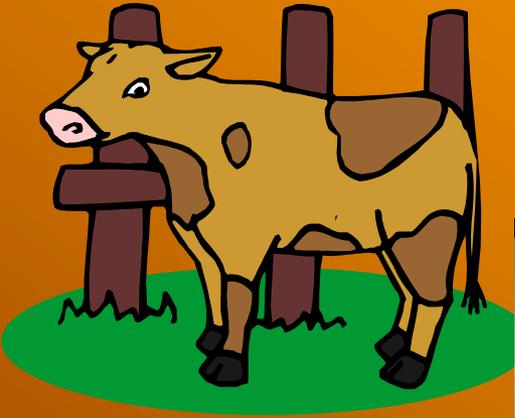


	Mac	T-Mac
Resistance to ≥ 2 antibiotics	18%	45%
Resistance to ≥ 3 antibiotics	13%	19%



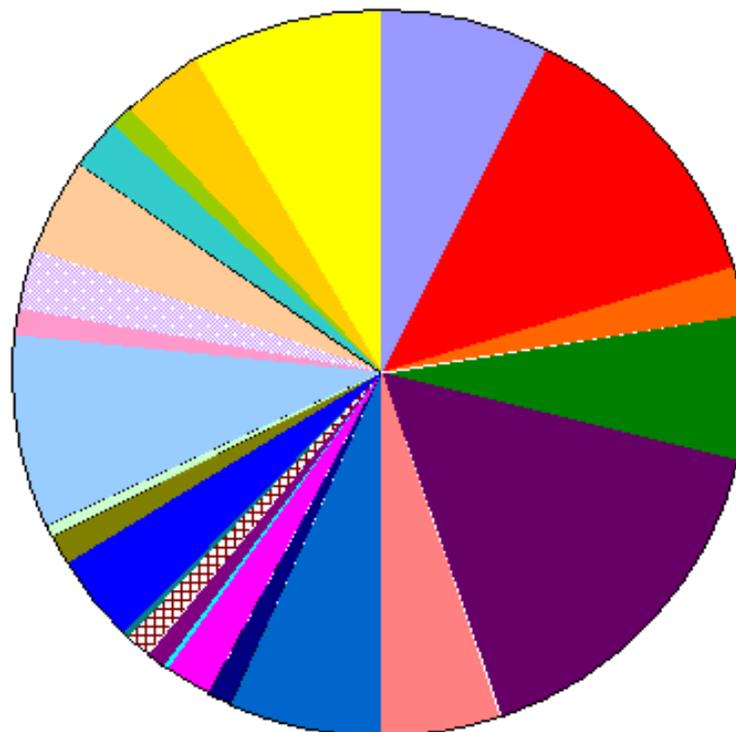
Fecal Metagenomic profiling of virulence and antibiotic resistance genes





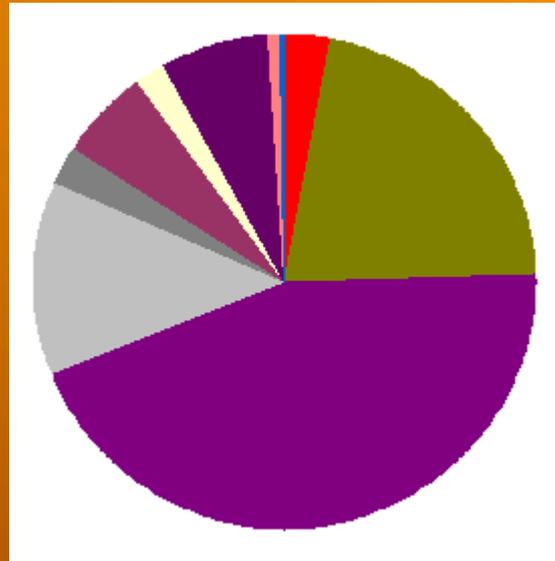
Bin genes by function

Distribution of functional protein groups in beef cattle feces



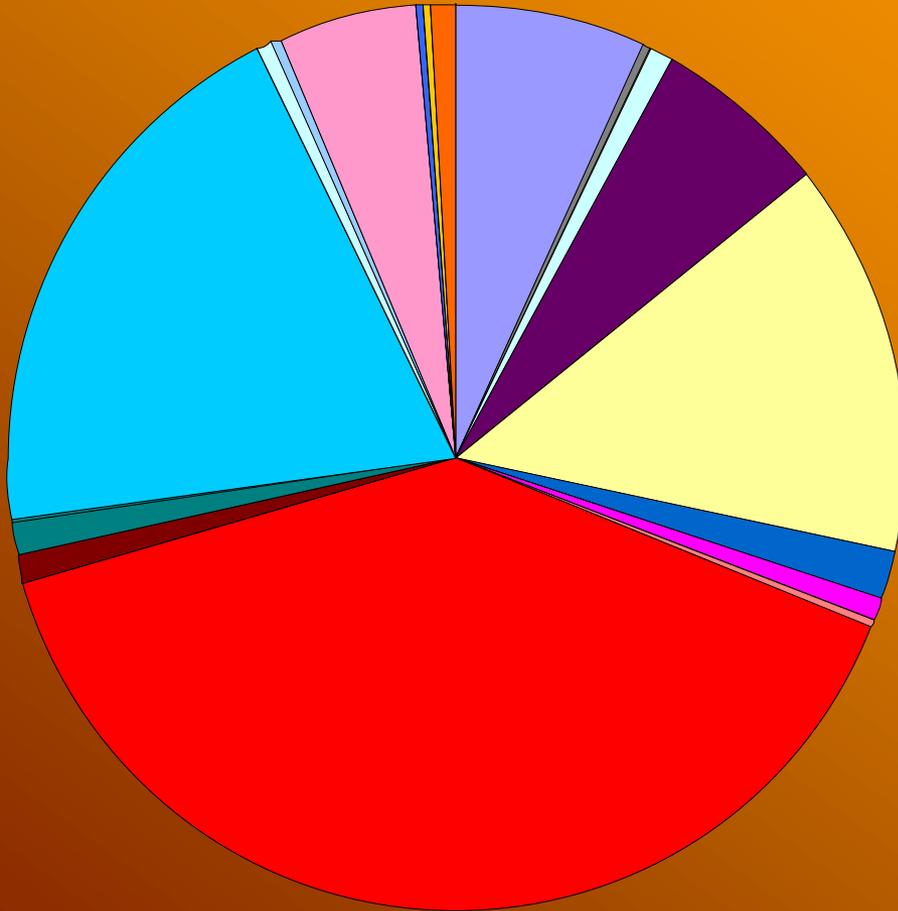
■ Amino acids and derivatives (7.38%)	■ Nucleosides and nucleotides (3.82%)
■ Carbohydrates (12.81%)	■ phosphorus metabolism (1.56%)
■ Cell division and cell cycle (2.25%)	■ Photosynthesis (<0.01%)
■ Cell wall and capsule (6.5%)	■ Potassium metabolism (0.53%)
■ Clustering-based subsystems (15.74%)	■ Prophage (0.07%)
■ Cofactors, vitamins, pigments (5.42%)	■ Protein metabolism (8.56%)
■ DNA metabolism (6.69%)	■ Regulation and cell signaling (1.12%)
■ Dormancy and sporulation (0.01%)	■ Respiration (2.85%)
■ Fatty acids and lipids (0.92%)	■ RNA metabolism (4.08%)
■ Macromolecular synthesis (0.02%)	■ Secondary metabolism (0.03%)
■ Membrane Transport (2.07%)	■ Stress response (2.17%)
■ Metabolism aromatic compounds (0.33%)	■ Sulfur metabolism (1.06%)
■ Miscellaneous (0.79%)	■ Unclassified (3.59%)
■ Motility and chemotaxis (0.92%)	■ Virulence (8.4%)
■ Nitrogen metabolism (0.32%)	

Virulence Genes



	Adhesion 3.18% (316)
	Type VI secretion systems 0.12% (12)
	Toxins and superantigens 0.03% (3)
	Type III, Type IV, ESAT secretion systems 21.16% (2104)
	Resistance to antibiotics and toxic compounds 44.49% (4423)
	Virulence 12.89% (1281)
	Pathogenicity islands 2.44% (243)
	Detection 0.09% (9)
	Prophage, Transposon 5.85% (582)
	Invasion and intracellular resistance 1.93% (192)
	Iron Scavenging Mechanisms 6.97% (693)
	Posttranslational modification 0.61% (61)
	Regulation of virulence 0.22% (22)

Cattle Feces

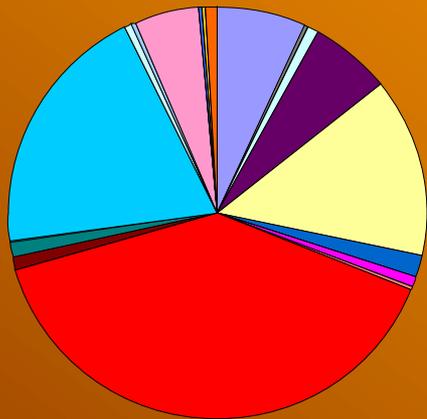


- Acriflavine resistance cluster
- Arsenic resistance
- Beta-lactamase
- Cobalt-zinc-cadmium resistance
- Integrons
- Mercury resistance operon
- MexE-MexF-OprN Multidrug Efflux System
- Multidrug Resistance Efflux Pumps
- Multidrug Resistance, G- Bacteria
- Resistance to fluoroquinolones
- Strep pneumoniae Vancomycin Tolerance Locus
- Teicoplanin-resistance in Staphylococcus
- The mdtABCD multidrug resistance cluster
- USS-DB-1
- USS-DB-4
- USS-DB-6
- Aminoglycoside adenylyltransferases
- Bacitracin Stress Response
- Mercuric reductase
- Methicillin resistance in Staphylococci
- Multidrug efflux pump in Campylobacter
- Multidrug Resistance, Gram-positive bacteria
- Multiple Antibiotic Resistance MAR locus
- Resistance to Vancomycin
- Streptothricin resistance
- Tetracycline resistance
- Tolerance to colicin E2
- USS-DB-2
- USS-DB-5
- Zinc resistance

44% of virulence genes are RATC

Cattle Feces

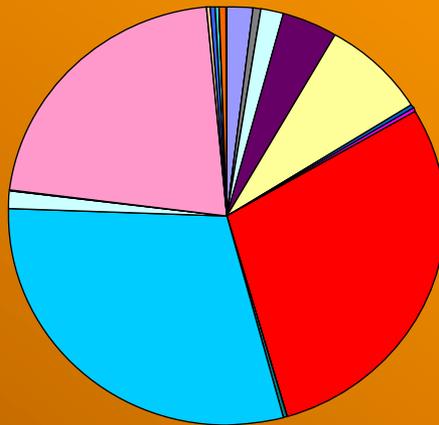
(n = 273,960 sequences)



44% of virulence genes are RATC

Cow Rumen

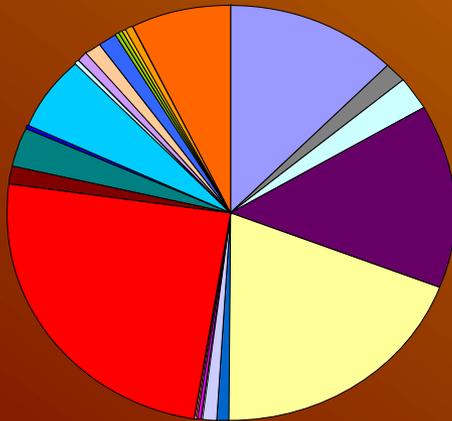
(n=264,849 sequences)



65% of Virulence are RATC

Farm soil

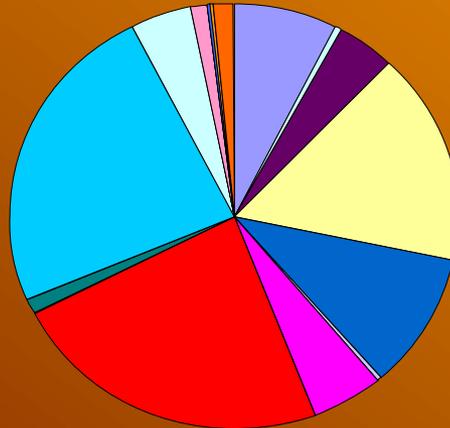
(n=138,347 sequences)



41% of virulence genes are RATC

Antartic Lake

(n = 100,085 sequences)



25% of virulence genes are RATC

- Acriflavin resistance cluster
- Arsenic resistance
- Beta-lactamase
- Cobalt-zinc-cadmium resistance
- Integrans
- Mercury resistance operon
- MexE-MexF-OprN Multidrug Efflux System
- Multidrug Resistance Efflux Pumps
- Multidrug Resistance, G- Bacteria
- Resistance to fluoroquinolones
- Strep pneumoniae Vancomycin Tolerance Locus
- Teicoplanin-resistance in Staphylococcus
- The mdtABCD multidrug resistance cluster
- USS-DB-1
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- Multidrug Resistance, Gram-positive bacteria
- Multiple Antibiotic Resistance MAR locus
- Resistance to Vancomycin
- Streptothricin resistance
- Tetracycline resistance
- Tolerance to colicin E2
- USS-DB-2
- USS-DB-5
- Zinc resistance

Metagenomic data can be used to quantify and track antibiotic resistance genes

-  Metagenomic data are quantitative (no PCR)
-  There are a large number of RATC classes represented in samples from widely different habitats
-  RATC profiles can be used to fingerprint samples
-  RATC gene profiles can be used to track relative amounts of antibiotic resistance genes over space and time

Research Interests - solving applied problems

Applied ecology of bacteria in agricultural production systems

Relationships of pathogens and fecal indicators

Fate and transport of antibiotic resistant bacteria and antibiotic resistant genes in manure, soil, and water

Characterizing "background" levels of bacteria and antibiotic resistance elements

Metagenomic profiling to link bacterial community structures and presence of virulence and antibiotic resistant genes

Industrial water re-use

Acknowledgements



Dan Miller



John E. Gilley



Chris Henry



Jaime LaBrie



Jennifer Frohner



Crystal Powers



Amy Mantz



Ryan McGee



Gilley Summer
Students



Alicia Craft

Acriflavine is a topical antiseptic. It has the form of an orange or brown powder. It may be harmful in the eyes or if inhaled. It is a dye and it stains the skin and may irritate. Commercial preparations are often mixtures with proflavine. It is known by a variety of commercial names. Acriflavine was developed in 1912 by Paul Ehrlich, a German medical researcher and was used during the First World War against sleeping sickness. It is derived from acridine. The hydrochloride form is more irritating than the neutral form. Acriflavine is also used as treatment for external fungal infections of aquarium fish. In recent years Acriflavine has been shown to have anti-cancer activity.

Diagram Functional Roles Subsystem Spreadsheet

Group Alias	Abbrev.	Functional Role	Reactions	Scenario Reactions	GO	Literature
all						
	USSDB1A	Hypothetical protein USSDB1A	-	-	-	none
	USSDB1B	Sugar ABC transporter, periplasmic sugar-binding protein USSDB1B	-	-	-	none
	USSDB1C	Sugar ABC transporter, sugar permease protein 1 USSDB1C	-	-	-	none
	USSDB1D	Sugar ABC transporter, sugar permease protein 2 USSDB1D	-	-	-	none
	USSDB1E	Hypothetical protein USSDB1E	-	-	-	none

Uptake Signal Sequence

Tetracycline_resistance,_ribosome_protection
_type 5.06% (224)

Results - antibiotic resistance profiles of pathogens

Ampicillin (AM 10)

Cefoxitin (FOX 30)

Amoxicillin (AMC 30)

Ceftriaxone (CRO 30)

Tetracycline (TE 30)

Streptomycin (S 10)

Ciprofloxacin (CIP 5)

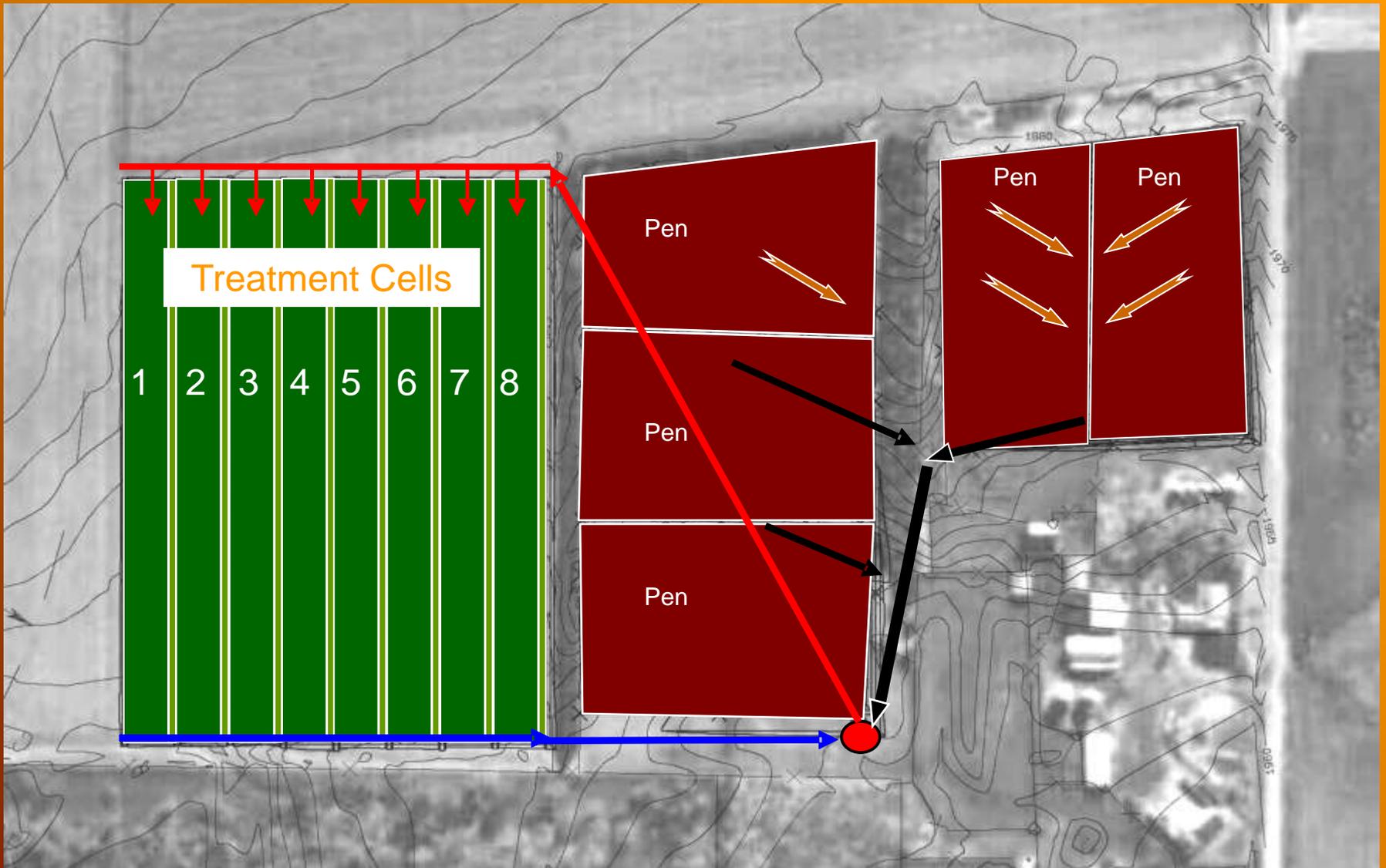
Kanamycin (K 30)

Nalidixic Acid (NA 30)

Sulfamethoxazole with Trimethoprim (SXT)

Chloramphenicol (C 30)

Gentamicin (GM 10)

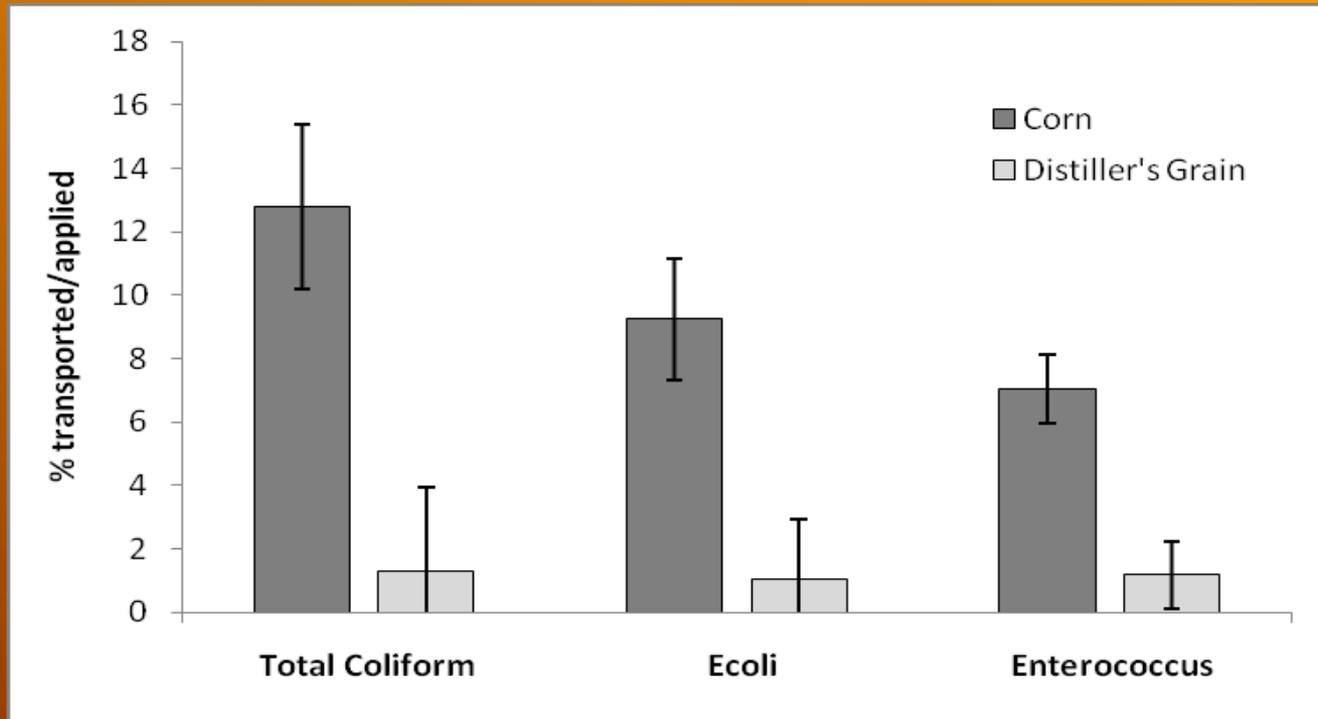


We have collected data this summer from three rain events...

273,900 reads passing quality filters

273,900 reads passing quality filters
Mean read length 494 bp

All manure is not created equal...



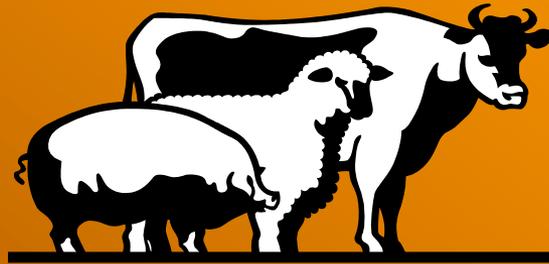
Percentage transport of Total Coliform, Ecoli, and Enterococcus in runoff as affected by diet. Microbial transport values were averaged across tillage and manure application rates. Vertical bars are standard errors.

Research Interests



My research focuses on characterizing how bacteria from animal manure travel through agricultural production systems, surface water and ground water. Currently, my main projects involve studying the fate and transport of pathogens, fecal indicator organisms, antibiotic resistant bacteria, and antibiotic resistance genes in runoff from feedlots, and runoff from manure-amended fields. I am also interested in DNA-based bacterial community profiling, and microbiological issues of industrial water re-use.

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