

[The kitchen sink]: Detecting and monitoring antibiotic resistance genes; ATAs and limiting foodborne pathogen carriage



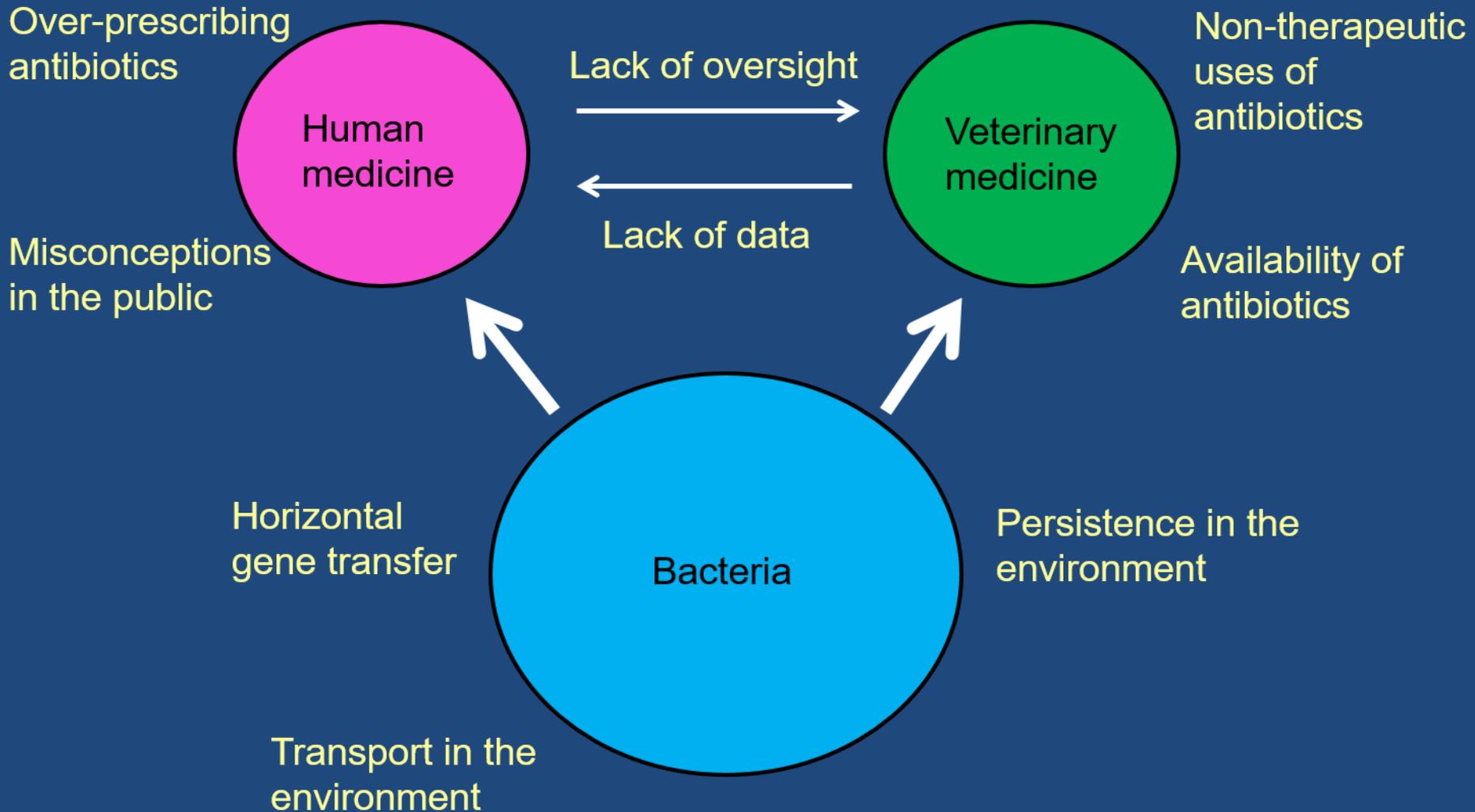
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Research Microbiologist, USDA, ARS, NADC

February 23, 2017

ARS/FSIS meeting

The antibiotic resistance problem: Who's fault is it?



Addressing the antibiotic resistance problem

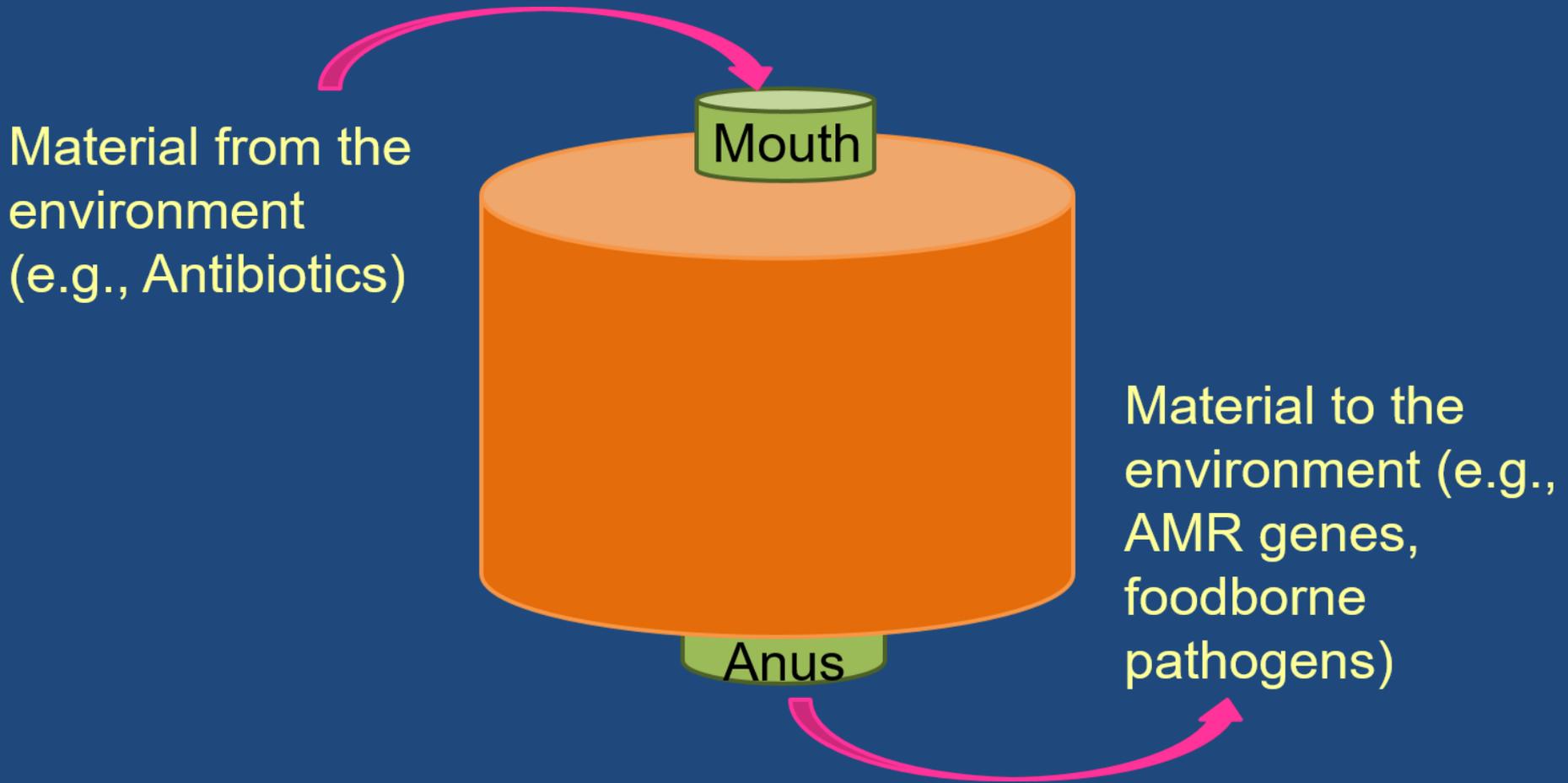
- Decrease the dissemination of antibiotic-resistant bacteria, including foodborne pathogens, in the environment and food chain
- Decrease the dissemination of antibiotic resistance genes among bacteria
- Decrease the selection pressure for resistance genes
 - Augment antibiotics
 - Decrease the need for antibiotic use
 - Alternatives to antibiotics

Environmental mitigation



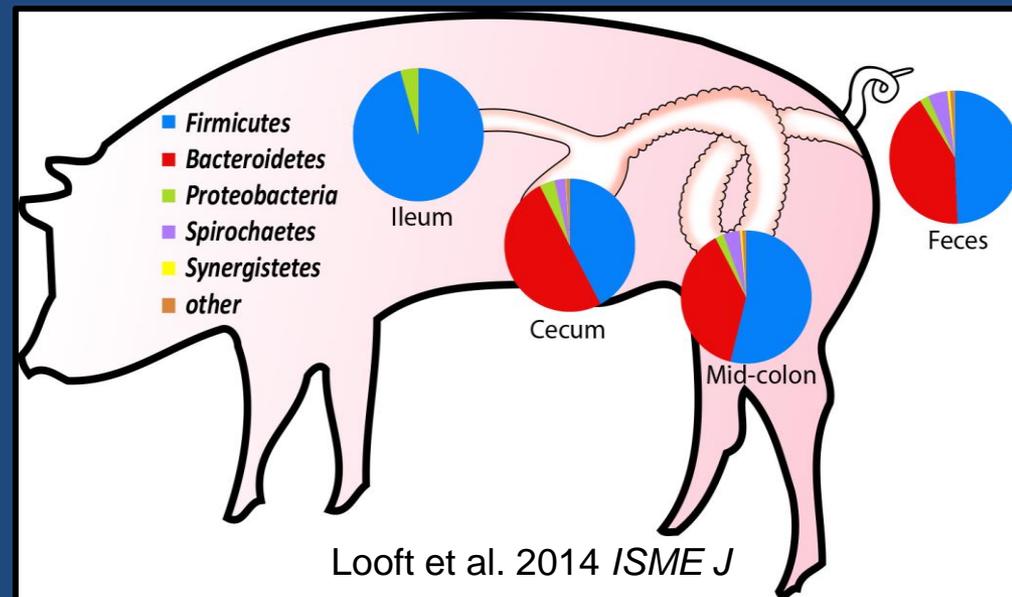
- Education and research
- Understand the ecology of antibiotic resistance genes in **ANIMAL** and environmental reservoirs

The gut: The environment of primary importance to AMR (1)

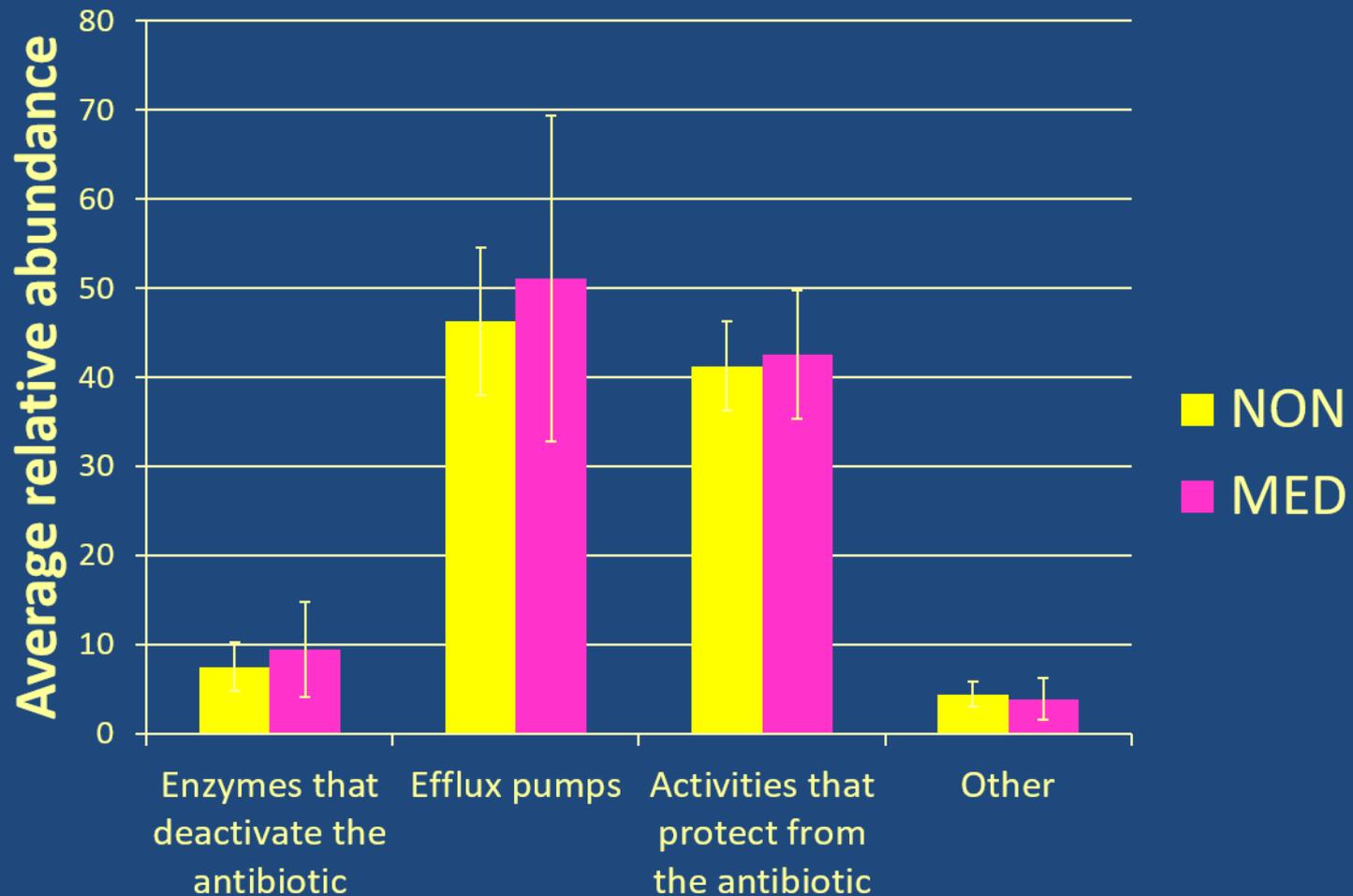


The gut: The environment of primary importance to AMR (2)

- >500 species in a mammalian gut, with certain subspecies being the important ecological unit
- Various states of the gut microbiota have been linked to health and disease
- Previous work from our lab showed over 100 different AMR genes in gut of non-medicated pigs



Swine microbiota harbor diverse antibiotic resistance genes regardless of antibiotic (ASP250) treatment



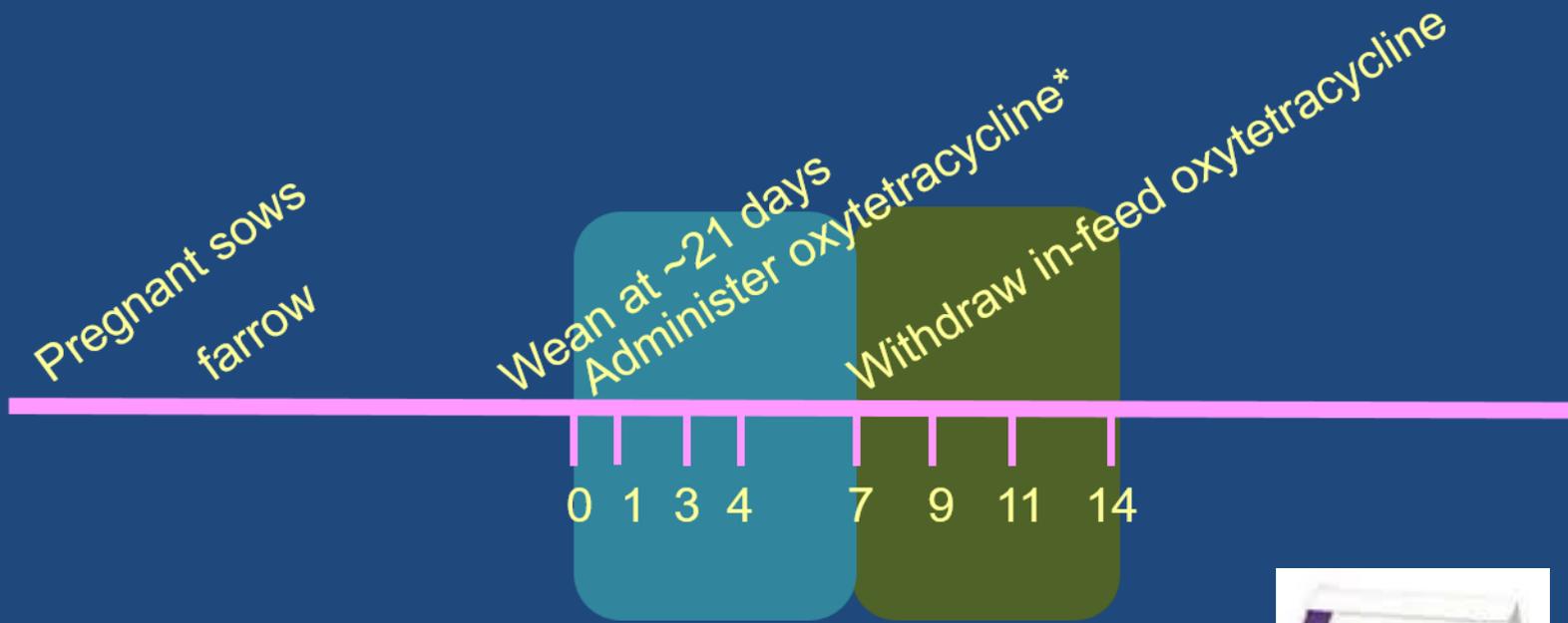
Gaps in AMR: #1

- Truth: Antibiotics are essential for the treatment of bacterial diseases in humans and livestock
- Truth: Antibiotic use selects for AMR evolution and transmission



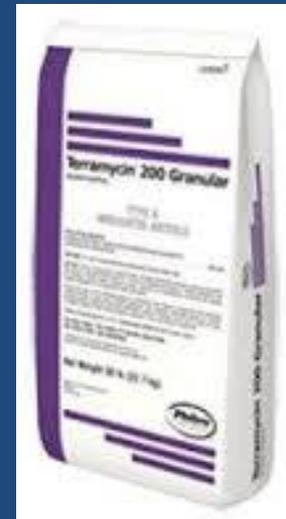
- Gap: Does route of antibiotic administration (oral vs. intramuscular) differentially impact the gut microbiota and antibiotic resistance gene carriage?

Study to address gap #1

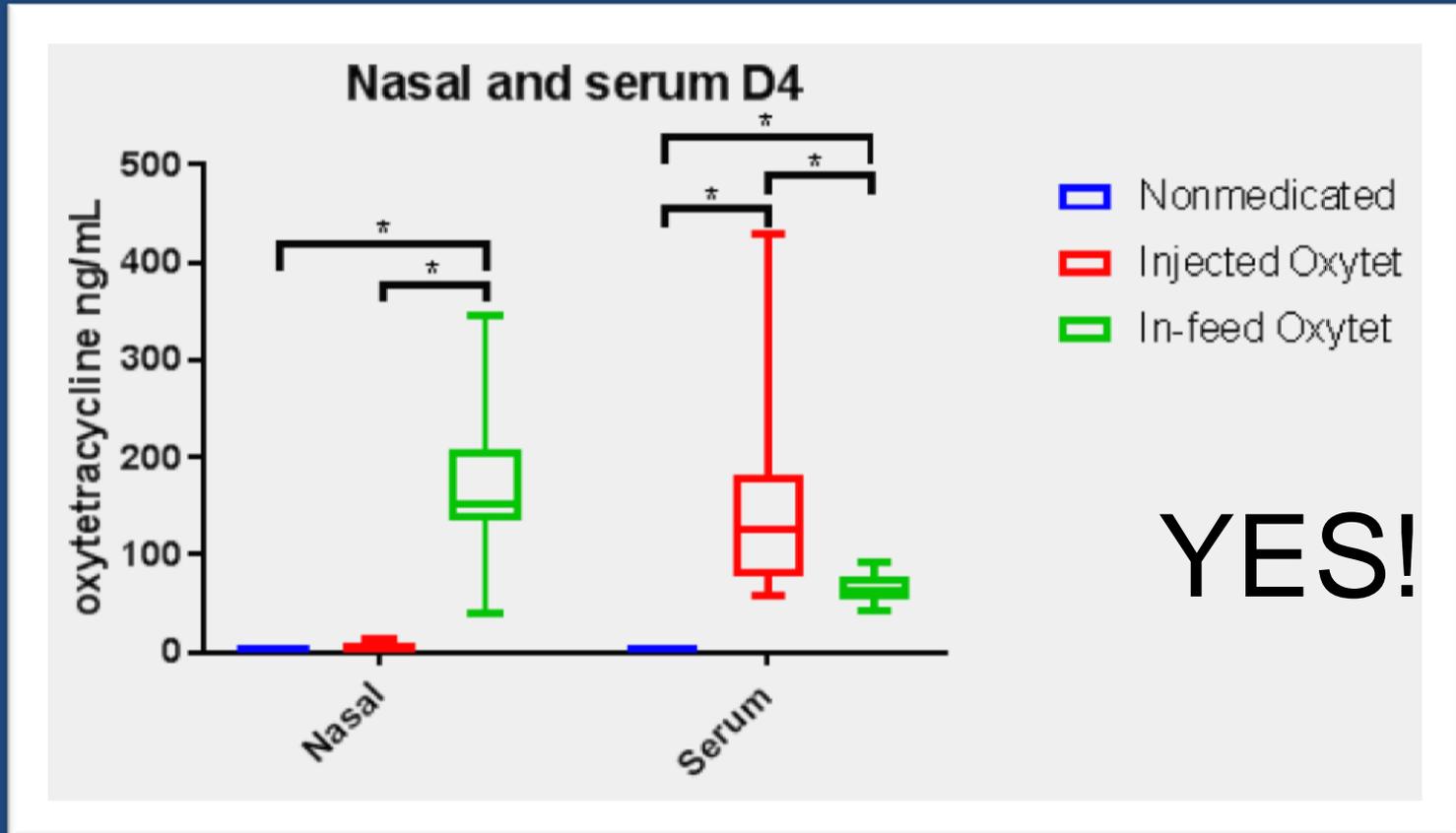


*Experimental groups:

- 1) Unamended starter feed
- 2) Unamended starter feed, intramuscular oxytetracycline on day 0
- 3) Starter feed containing Terramycin 200



Did route of administration impact antibiotic concentration in the pigs?

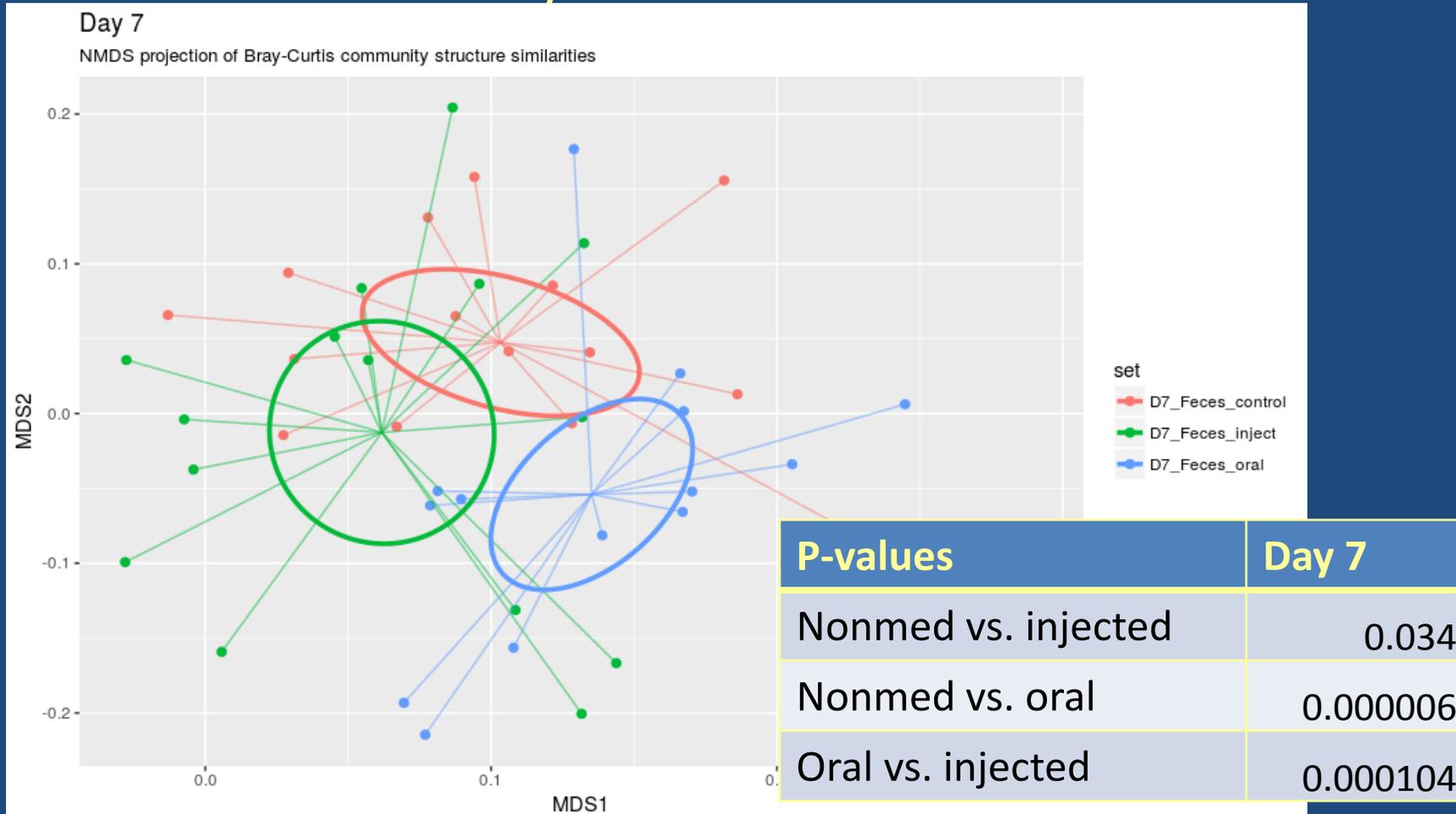


Did route of antibiotic administration differentially impact the microbiota? (1)

P-values	Day 0
Nonmed vs. injected	0.58
Nonmed vs. oral	0.29
Oral vs. injected	0.99

Did route of antibiotic administration differentially impact the microbiota? (2)

- MDS ordination of day 7 fecal microbiotas

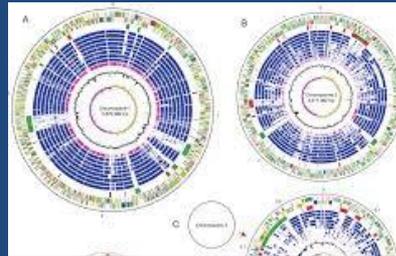


Conclusions, part 1

- Differential routes of oxytetracycline administration lead to different antibiotic concentrations in the body
 - Follow-up study will also evaluate disease challenge
- Intramuscularly administered oxytetracycline does not shift the microbiota as dramatically as in-feed oxytetracycline
 - AMR genes yet to be evaluated

Gaps in AMR: #2

- Truth: Surveying antibiotic resistance gene dissemination is important
- Truth: It is difficult to determine which genes to survey



- Gap: Can we develop a high-throughput sequencing method that is more targeted than metagenomics or WGS but more permissible than PCR?

Direct sequencing of hundreds of AMR amplicons simultaneously

- Technology development: Diverse Antibiotic Resistance and Transfer Elements-Quantitative Monitoring
 - A high-throughput DNA sequencing method to sequence hundreds of resistance genes, mobile genetic elements and phylogenetic markers
 - Applications for large-scale monitoring and determining efficacy of mitigation strategies

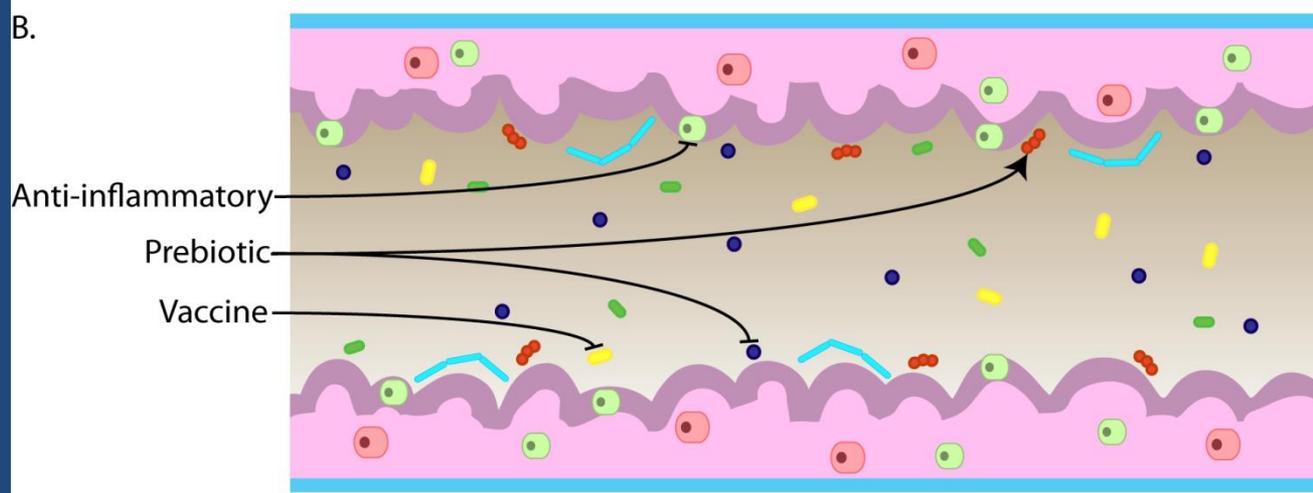
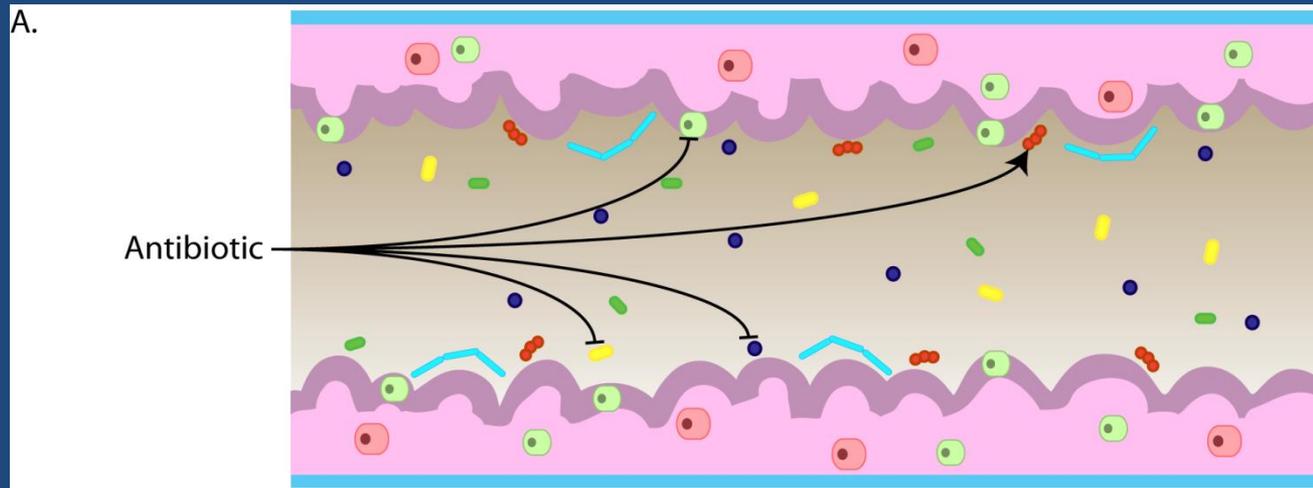
ISU
Adina Howe
Michelle Soupir



USDA
Heather Allen
Tom Moorman
Grinnell College
Shannon Hinsa

Evaluating alternatives (1)

- How do certain alternatives to antibiotics modulate the swine immune system and gut microbiota, and is health improved?



Evaluating alternatives (2)

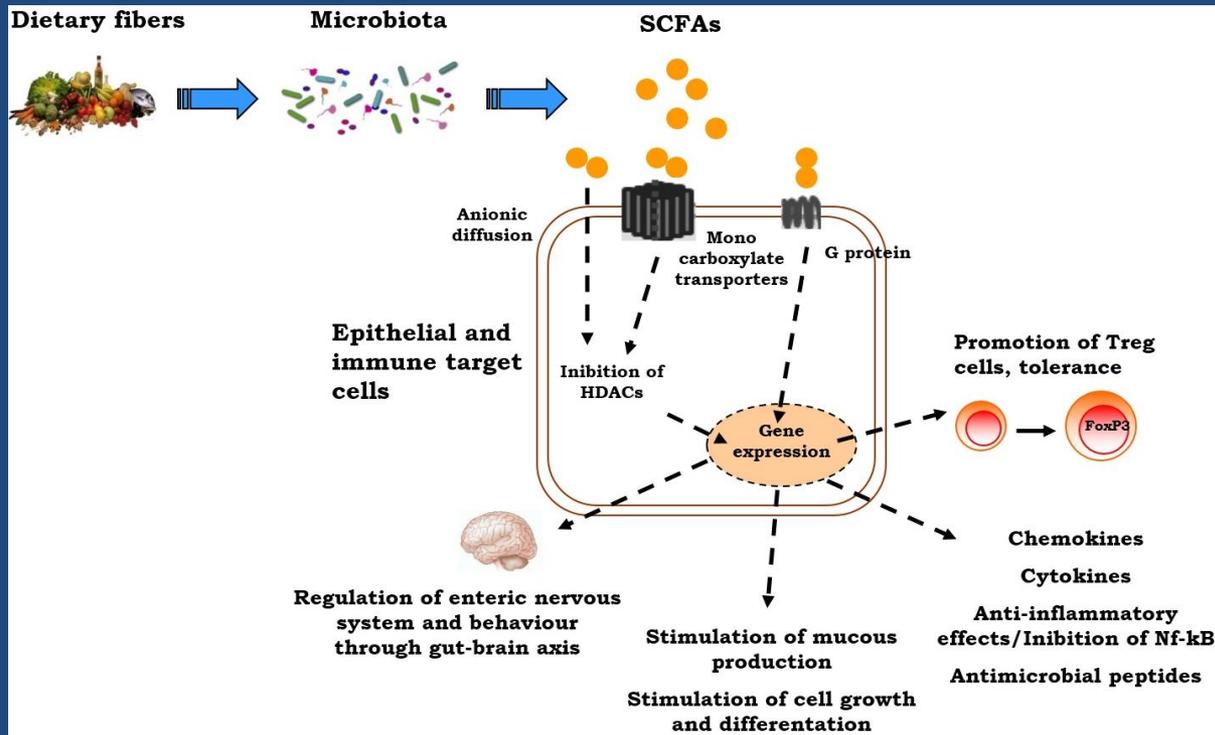
- How do certain alternatives to antibiotics modulate the swine immune system and gut microbiota, and is health improved?
- Modulating butyrate-producing bacterial communities: resistant [potato] starch (RPS)



Bacterial fermentation products can alter immune status

- Resistant starch

- Enhances the growth of beneficial bacteria that produce short-chain fatty acids (SCFAs) such as butyrate



Butyrate-producing bacteria in swine

Eubacterium nodatum

Megasphaera elsdenii LC-1

Butyricoccus pullaceacorum

Peptoniphilus gorbachii

Intestimonas butyriciprudecens

Anaerostipes hadrum A

Anaerostipes hadrum B

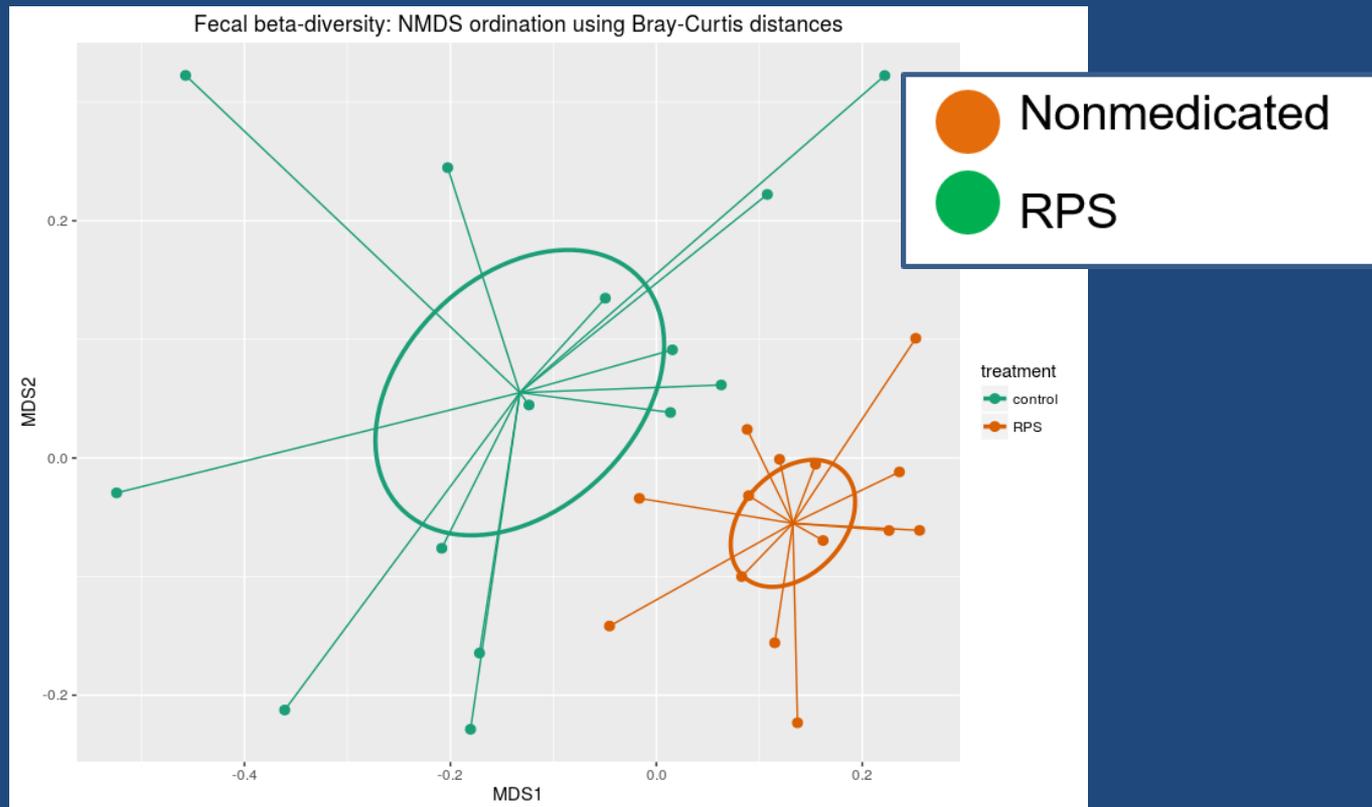
Roseburia hominis

Roseburia faecis

Levine et al. 2013 *Appl Environ Microbiol*
 Trchsel et al. 2016 *Appl Environ Microbiol*

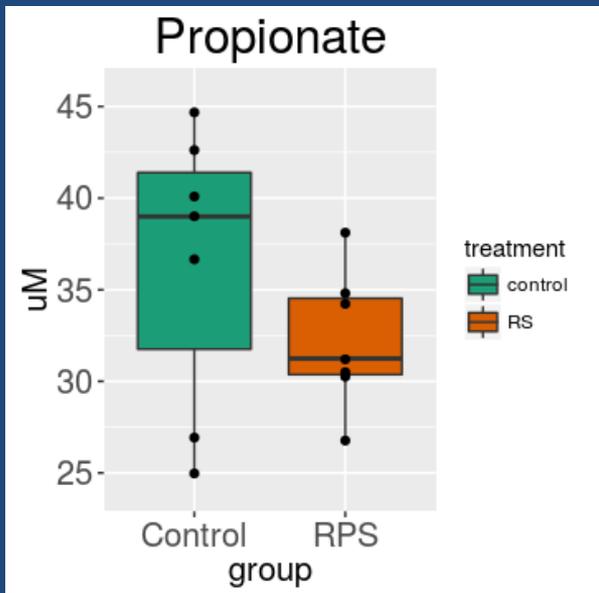
RPS changes the bacterial diversity (16s rRNA gene sequence)

Each data point represents the microbial community of one pig

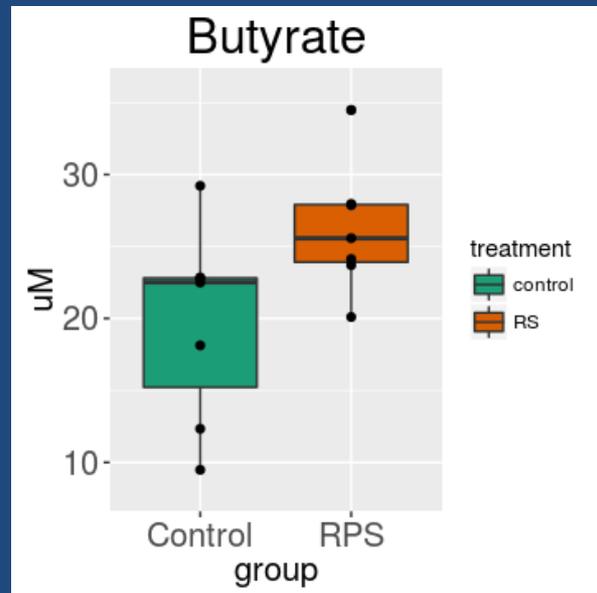


PERMANOVA: $p=2e-6$; $F=8.98$

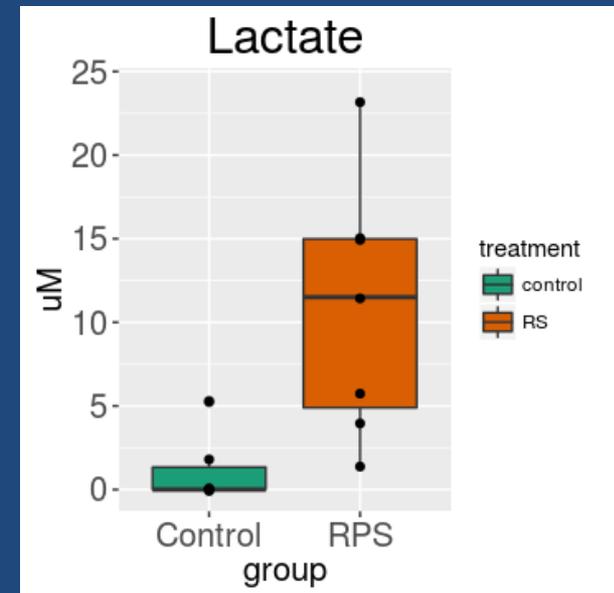
RPS alters cecal SCFA profiles



$p = 0.2$

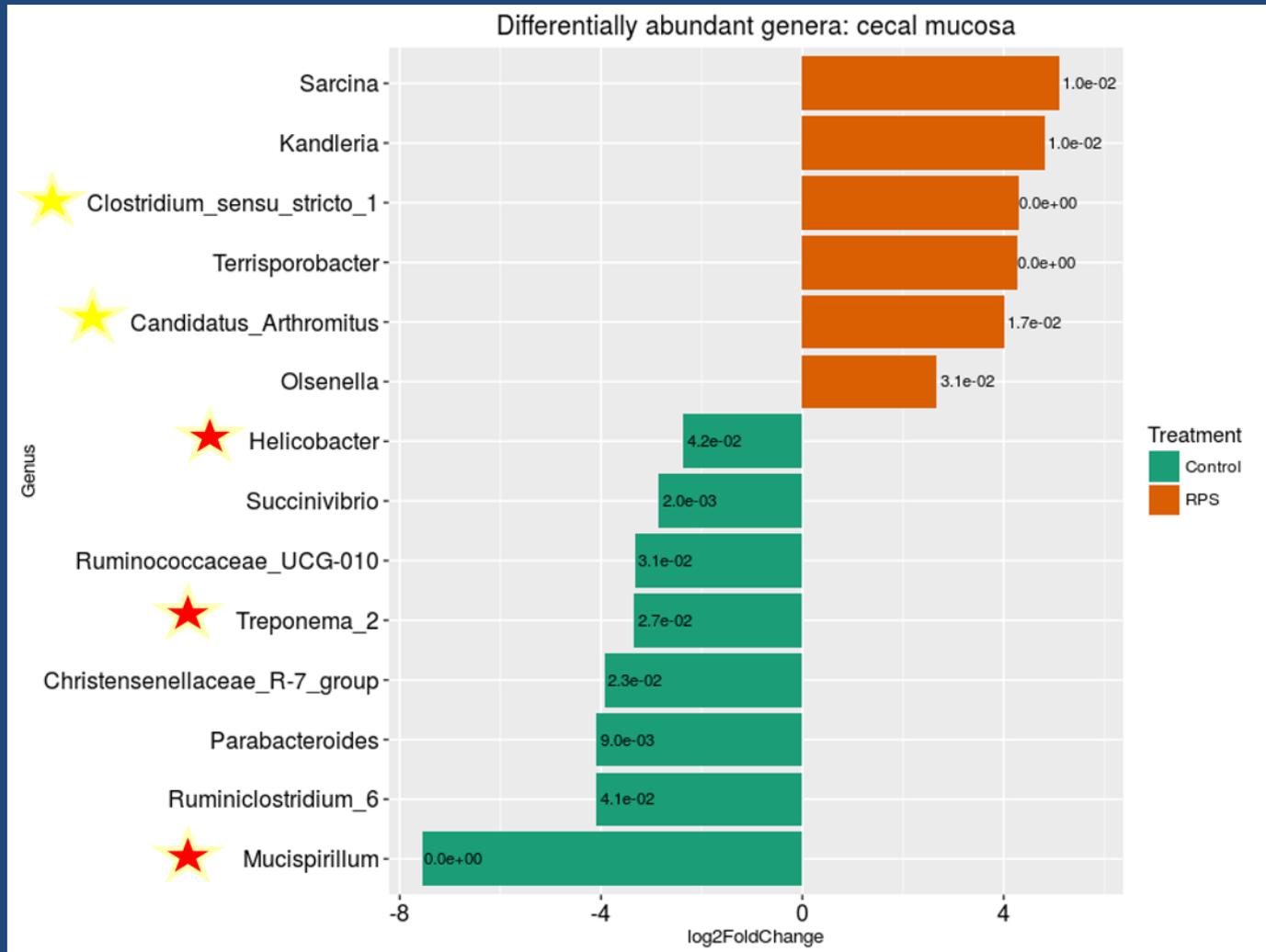


$p = 0.05$



$p = 0.01$

Differentially abundant bacteria along the cecal mucosa



Modulation of host mucosa

- Changes in gene expression along the cecal mucosa
- Changes in cecal T-cell populations
- Next steps: Evaluate effects of RPS on host and microbiota in context of *Salmonella* challenge

Conclusions, part 2

- Inclusion of 5% raw potato starch in piglet diets causes significant changes:
 - Increased butyrate production
 - Altered microbial community
 - Reduced niche for facultative aerobic potential pathogens
 - Improved epithelial barrier function
- **Raw potato starch may help ease the stress of weaning and reduce prophylactic antibiotic use**



Reducing foodborne pathogens in the food chain

- Preharvest control of *Campylobacter* in turkeys
 - Develop efficacious vaccine
- Evaluate effects of industry-relevant antibiotics in turkeys (bacitracin methylene disalicylate (BMD))
 - On AMR and microbiota

Torey Looft
Matthew Sylte

Reducing foodborne pathogens in the food chain (1)

- Preharvest control of STEC (*E. coli* O157:H7) in cattle
 - Develop efficacious vaccine
 - Identify bacteria in the rectoanal junction (RAJ) and/or fecal microbiome with probiotic potential.
 - Identify bacterial ligands and tissue receptors involved in STEC adherence to the RAJ to develop “blocking” analogs

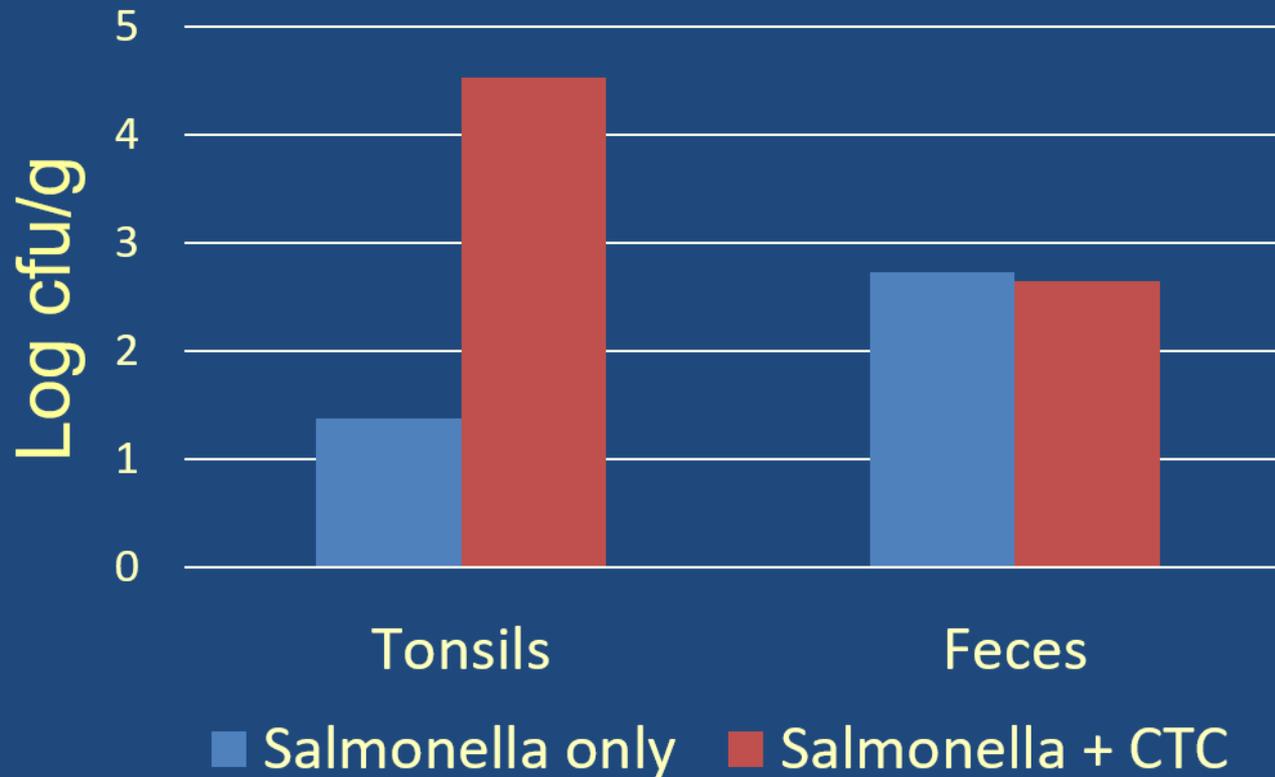
Indira Kudva
Vijay Sharma

Reducing foodborne pathogens in the food chain (2)

- Preharvest control of *Salmonella* in swine
 - Development of cross-species, cross-protective DIVA vaccine (patented)
 - Identification of potential gut microbiota status that yields decreased *Salmonella* shedding
 - Identification of the effects of antibiotics on multidrug-resistant (MDR) *Salmonella* Typhimurium virulence
 - Analysis of monophasic *Salmonella* transmission and shedding*

Shawn Bearson
Brian Brunelle

Chlortetracycline administration to swine increased MDR *Salmonella* colonization in the tonsil but not feces



Shawn Bearson
Brian Brunelle
Devin Holman



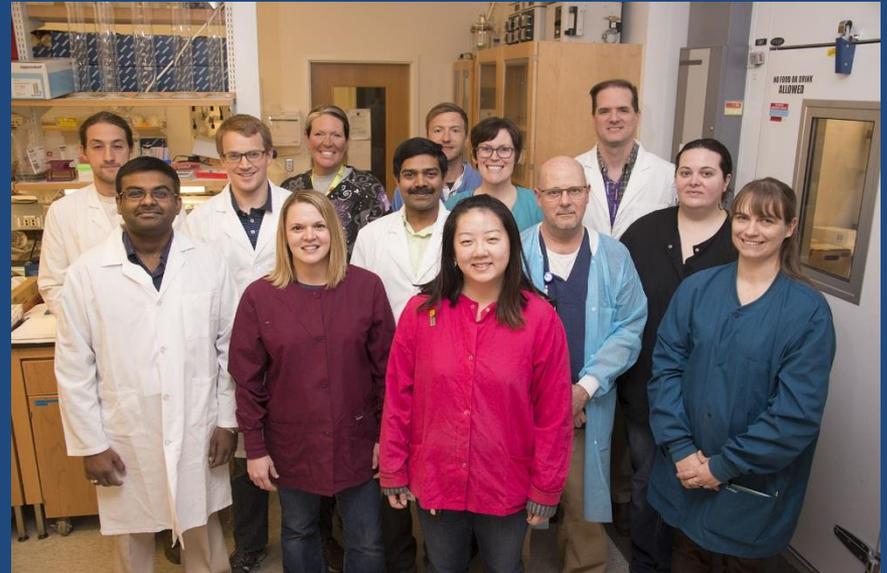
Acknowledgements

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- Mike Marti and Jim Fosse
- Darrell Bayles and David Alt

ISU

- Phil Colgan
- Adina Howe

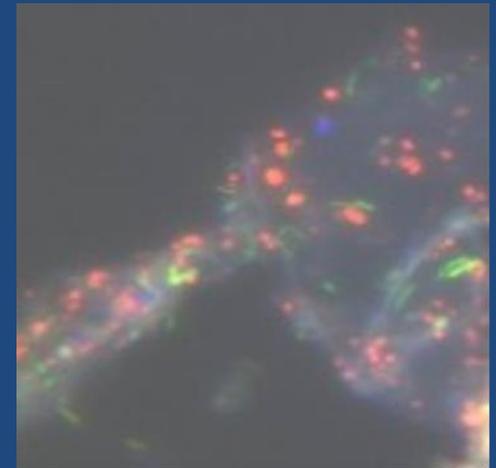


Gaps in AMR: #2

- Truth: Colistin is a drug of last resort for certain human infections.
- Truth: Colistin is not used in animal agriculture in the U.S., but it is in China and other countries
- Truth: The first colistin-resistant *E. coli* infection in a human was reported last year in the U.S.
- Gap: How was that resistance gene selected (*mcr-1*), and how did it get here?

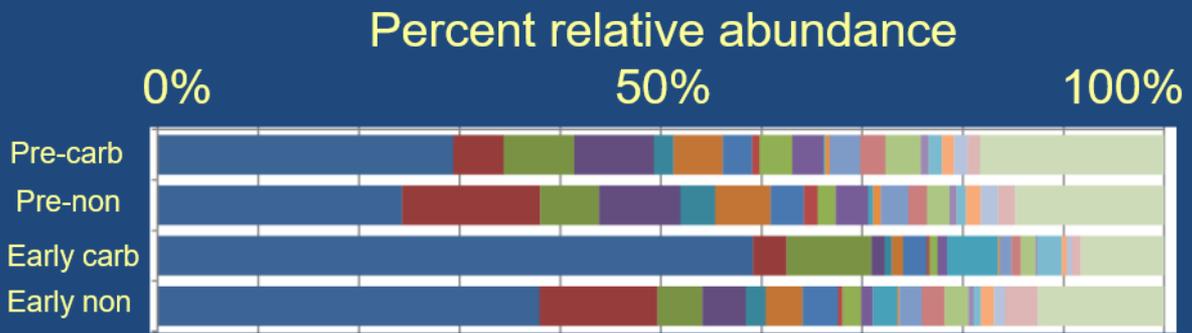
Measuring the microbiota

- Culture the bacteria
 - $\sim < 1\%$ cultivable by standard laboratory techniques
- Sequence bacterial DNA (culture-independent)
 - Genomics
 - Metagenomics
 - **16S rRNA gene sequence analysis**



Walker et al 2008
*Environmental
Microbiology*

Beyond relative abundance



Are *Prevotella* populations increasing in abundance with carbadox treatment? No.

	Non-medicated	Carbadox
dPCR <i>Prevotella</i> counts (LOG ₁₀)	9.8, SE = 0.26	10.0, SE= 0.11