

Intestinal Microbiota and Metagenomics: Tools to Improve Animal Health & Performance

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NATIONAL STRATEGY FOR COMBATING ANTIBIOTIC- RESISTANT BACTERIA

Vision: The United States will work domestically and internationally to prevent, detect, and control illness and death related to infections caused by antibiotic-resistant bacteria by implementing measures to mitigate the emergence and spread of antibiotic resistance and ensuring the continued availability of therapeutics for the treatment of bacterial infections.

September 2014



<http://www.whitehouse.gov/the-press-office/2014/09/18/executive-order-combating-antibiotic-resistant-bacteria>



Intestinal Microbiota and Metagenomics: Tools to Improve Animal Health & Performance

Researchable topics:

- How to feed stressed animals?
- How to manage microbiomes of mucosal surfaces to maximize health & performance?
- Do crop production practices impact nutrients, microbiomes & animal health?

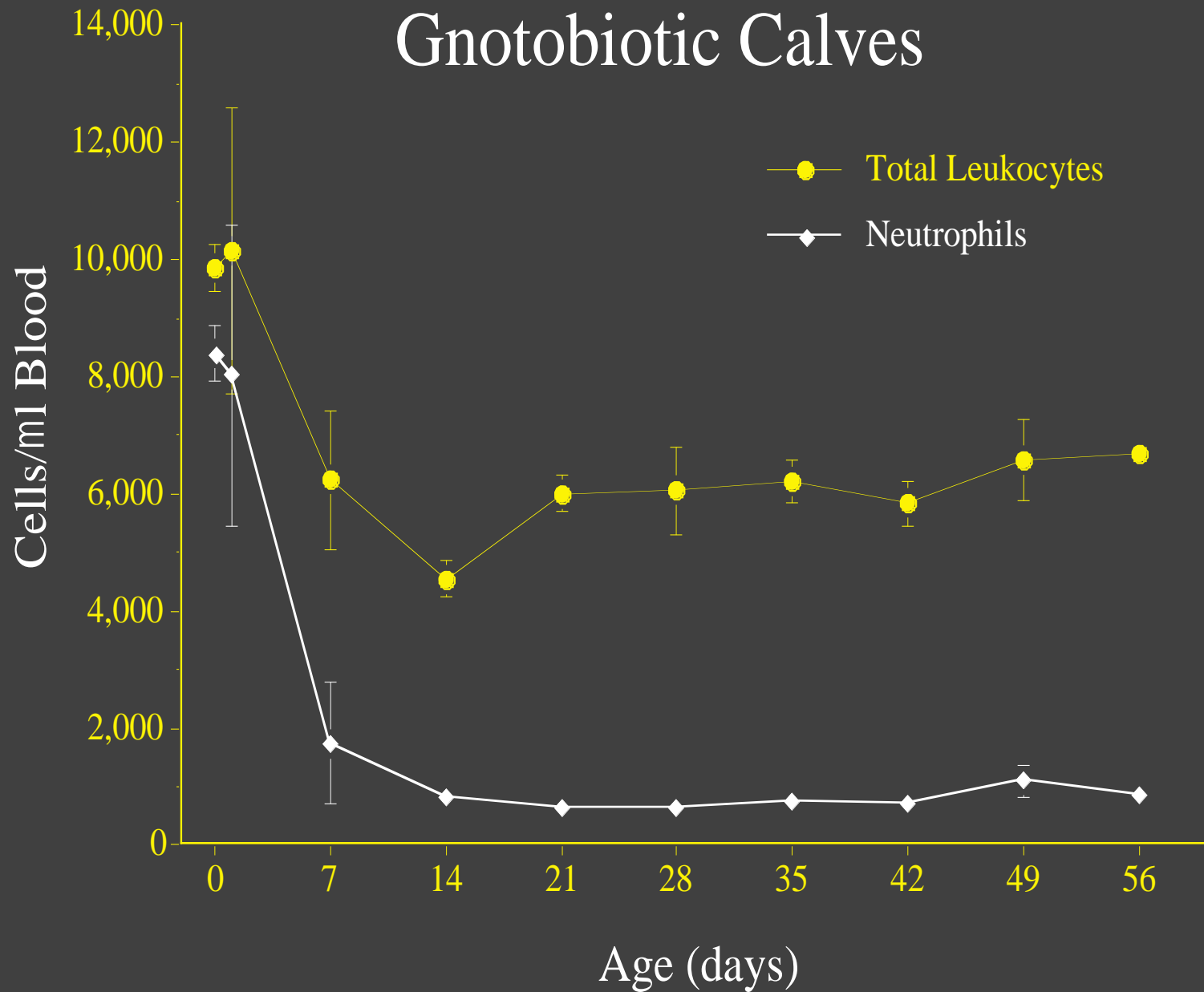
Big Picture: Veterinary Vaccine Opportunities

- Agriculture represented a \$395 B industry in 2012
 - ◆ Crops 53.8%
 - ◆ Livestock/Poultry 46.2%
- Finite research funding resources
 - ◆ Focus on biggest impact on animal protein production
- Most costly diseases to livestock producers
 - ◆ Respiratory diseases
 - ◆ Enteric diseases
 - ◆ Reproductive diseases
 - ◆ Mastitis
- Stressed animals have highest incidence of disease
 - ◆ Often predisposes each of the diseases above

Political realities

- | Although *in feed* antibiotics are recommended to help maintain a healthy digestive system and enhance performance, fear and politics have combined to forbid use of important antibiotics in some regions.
- | This pressure is not likely to go away.
- | Are immune modulators an alternative to *in feed* antibiotics?

Gnotobiotic Calves



Effect of normal flora?

- | It appears the absence of an intestinal flora reduces the need for “normal” circulating neutrophil numbers.
- | Is this one mechanism by which *in feed* antibiotics enhance performance?
- | Do *in feed* antibiotics help reduce nutrient demands of a host by reducing the energy expenditure of the immune system elicited by normal flora?

Immune system nutritional costs?

Caloric and protein demands of immunity

- Leukocyte production - part of host maintenance
PMNs have 6-9 hr half-life ($\sim 3.3 \times 10^{11}$ /day)
- Leukocyte activities - protein production (enzymes, receptors, Ig, cytokines, antibacterial peptides, etc.), cytotoxic activities, surveillance
- Leukocyte products can alter host metabolism by raising the BMR via febrile responses and increased catabolism of protein (e.g., TNF)

What are some numbers?

Caloric and protein demands of immunity

- Humans suffering from severe infection causing sepsis, resting energy expenditure increased progressively over 7 d to ~40% above normal and remained elevated 3 weeks from the onset of illness. Over a 3-week period patients lost 13% of their total body protein (Plank and Hill, 2000).

Bovine numbers on demands of immunity

- Maintenance energy for a 600 kg dairy cow ~9.7 Mcal Net energy/d
- If increase energy expenditure 40% to mount inflammatory response the energy requirement increases by nearly 4 Mcal/day
- Requires cow consume an additional 2.4 kg of diet (assuming a diet that provided 1.65 Mcal NE_L/kg). (Jesse Goff, USDA-NADC)

What are the costs?

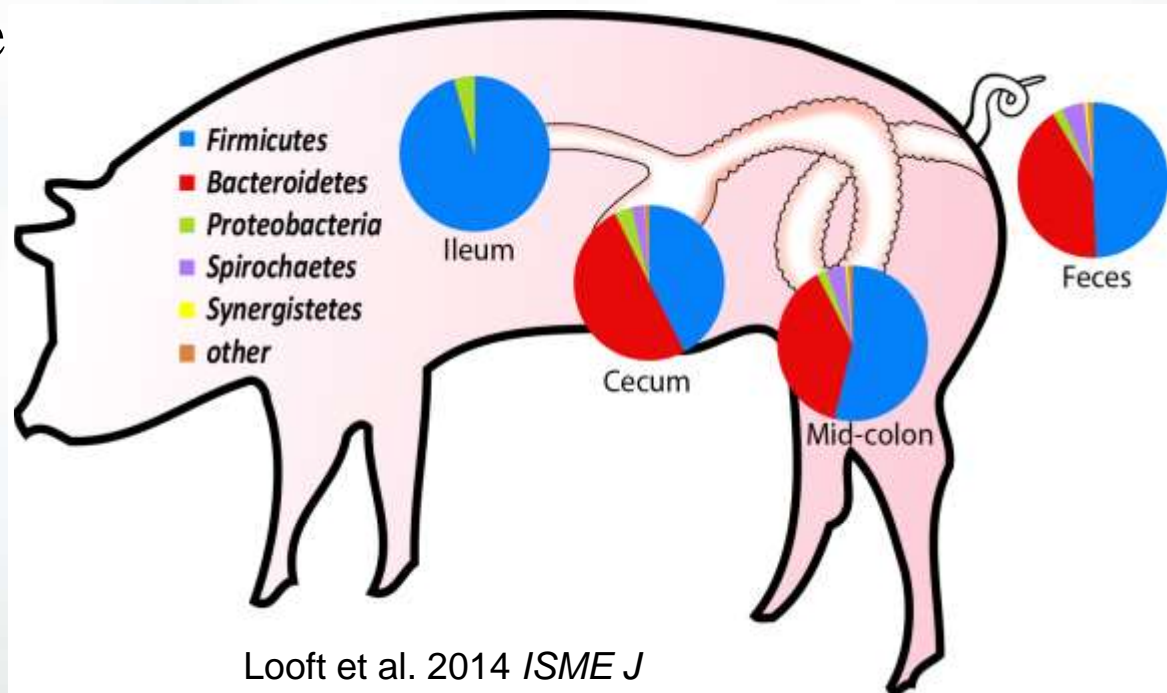
- What is the cost of a low grade fever running through a pen of livestock?
- What is the risk cost of one diseased animal to the rest of its pen-mates? How do we estimate this?
- If this cost is real, how do we value a product that reduces this risk?

How to manage microbiomes of mucosal surfaces to maximize health status?

- Microbiome of mucosal surfaces plays huge role in health and immune status of animals:
 - ◆ What is an optimal microbiome for livestock and poultry?
 - ◆ Changes over animal's life?
 - ◆ How does the microbiome interact with host defenses?
 - ◆ How does diet impact the intestinal microbiome?
 - Impact of diet changes

Complex microbial communities: gastrointestinal microbiota

- >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
- Feces only partially represents the upstream communities



With what do you modulate the gut microbiota?

- Probiotics
- Prebiotics
- Synbiotics
- Others???



Promote health!
Prevent disease!

 Vet Recommended



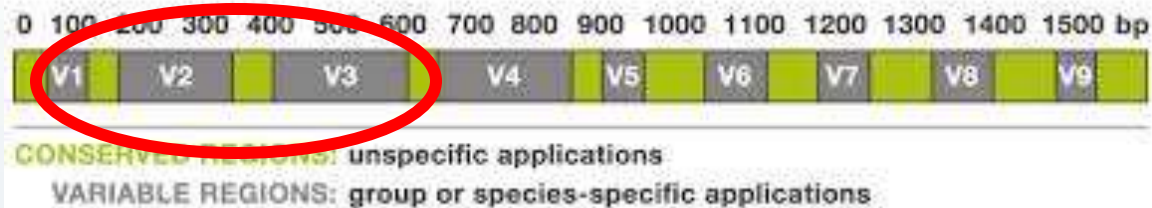
Microbiota analyses

How it's done and what you can learn from it

Heather K. Allen, PhD
Research Microbiologist
Food Safety and Enteric Pathogens Research Unit

Microbial diversity analyses

- The 16S rRNA gene is universal across bacteria
- Has both constant and variable regions
- Sequence variable regions to determine bacterial membership
- Compare across environments, treatments, etc.



MiSeq amplicon protocol (Schloss SOP)

- Use PCR to amplify 16S rRNA genes from intestinal samples using MiSeq amplicon primers

Fecal DNA extractions in 96-well format via robotics



- Significant because...
 - ◆ DNA in 96-well format
 - ◆ Amplicon PCRs in 96-well format
- High-throughput capacity!

Positive control: “mock” community

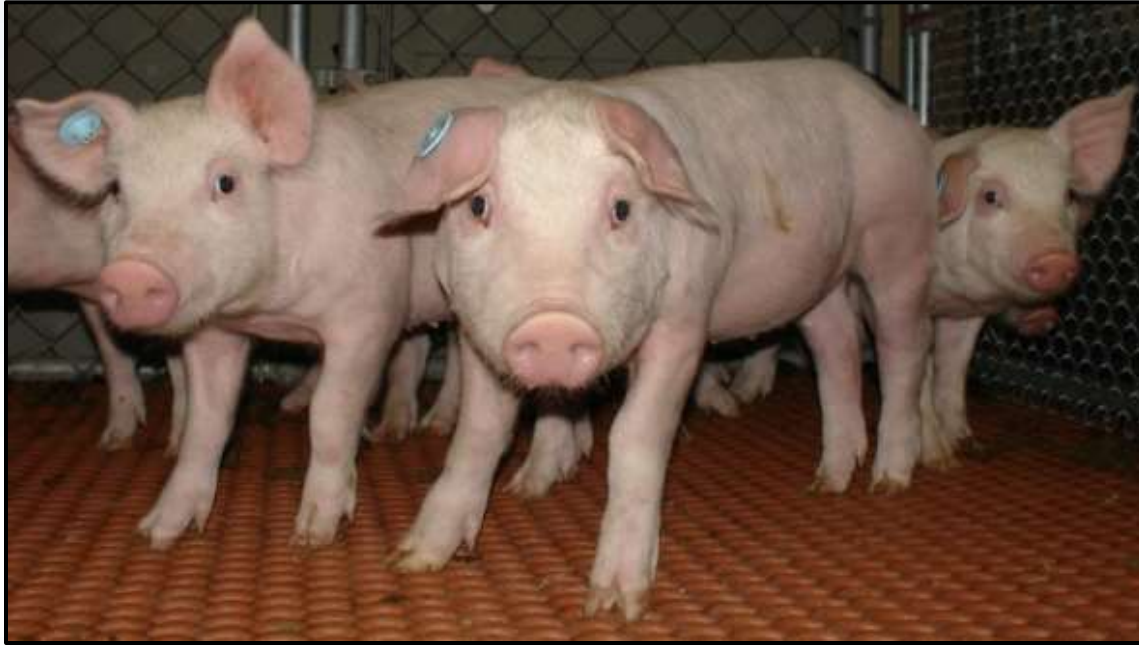
- Purposes
 - ◆ Test the error rate of 16S analysis pipeline
 - ◆ Partially assess our ability to detect known swine-associated bacteria

Positive control: “mock” community

<i>Campylobacter jejuni</i>
<i>Salmonella enterica</i> serovar Typhimurium
<i>Escherichia coli</i>
<i>Megasphaera elsdenii</i>
<i>Cloacibacillus porcorum</i> CL-84
<i>Brachyspira hyodysenteriae</i>
<i>Haemophilus parasuis</i> (29755)
<i>Bordetella bronchiseptica</i> (1289)
<i>Staphylococcus aureus</i> (USA300)
<i>Bacteroides thetaiotamicron</i>
<i>Methanobrevibacter smithii</i>
<i>Faecalibacterium prausnitzii</i> (A2-165)
<i>Streptococcus parasanguinis</i>
<i>Parabacteroides merdae</i>
<i>Oscillibacter valericigenes</i>
<i>Desulfovibrio gigas</i>
<i>Lactobacillus delbruekii</i> subspecies <i>bulgaricus</i>
<i>Coriobacterium glomerans</i>
<i>Roseburia hominis</i> (A2-183)
<i>Oxalobacter formigenes</i> (BA-2)

20
different
species

Swine gut microbial ecology



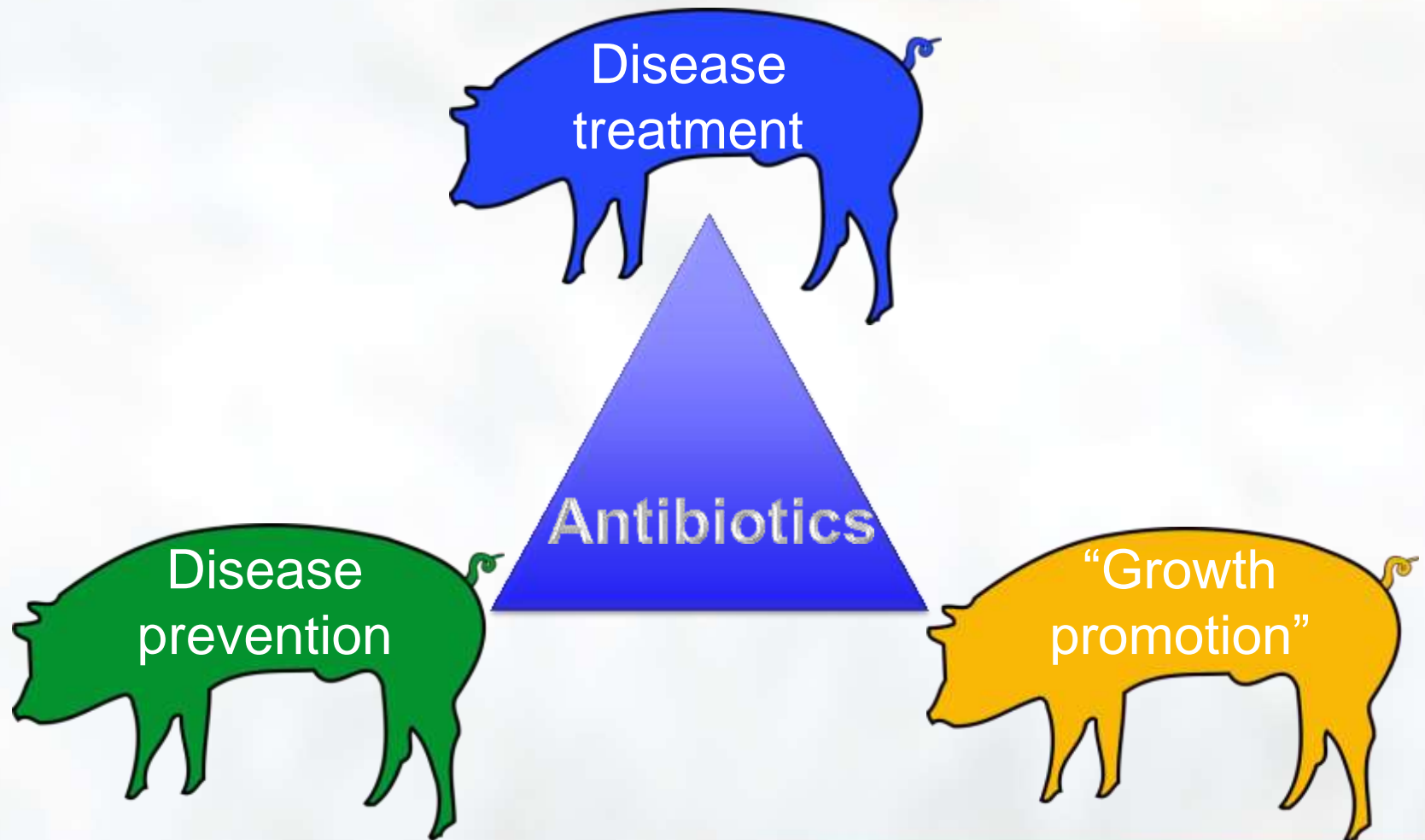
- Swine become pork
 - ◆ Improve swine health
 - ◆ Improve food safety

Objectives

- ◆ Define the effects of disturbances, such as in-feed antibiotics and *Salmonella* challenge, on gut bacteria and phages
 - *Collateral effects*
 - *Beneficial effects*
- ◆ Investigate alternatives to agricultural antibiotics



Challenges of agriculture antibiotic alternatives



Antibiotic alternatives



Target specific pathogens

- Vaccines
- Phage therapy
- Bacteriocins
- Predatory bacteria

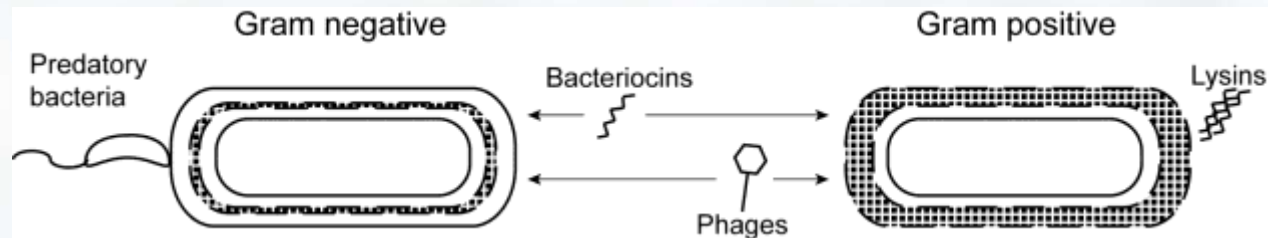


Modulate the gut microbiota

- Competitive exclusion (e.g. fecal transplant therapy)
- Probiotics/prebiotics/synbiotics

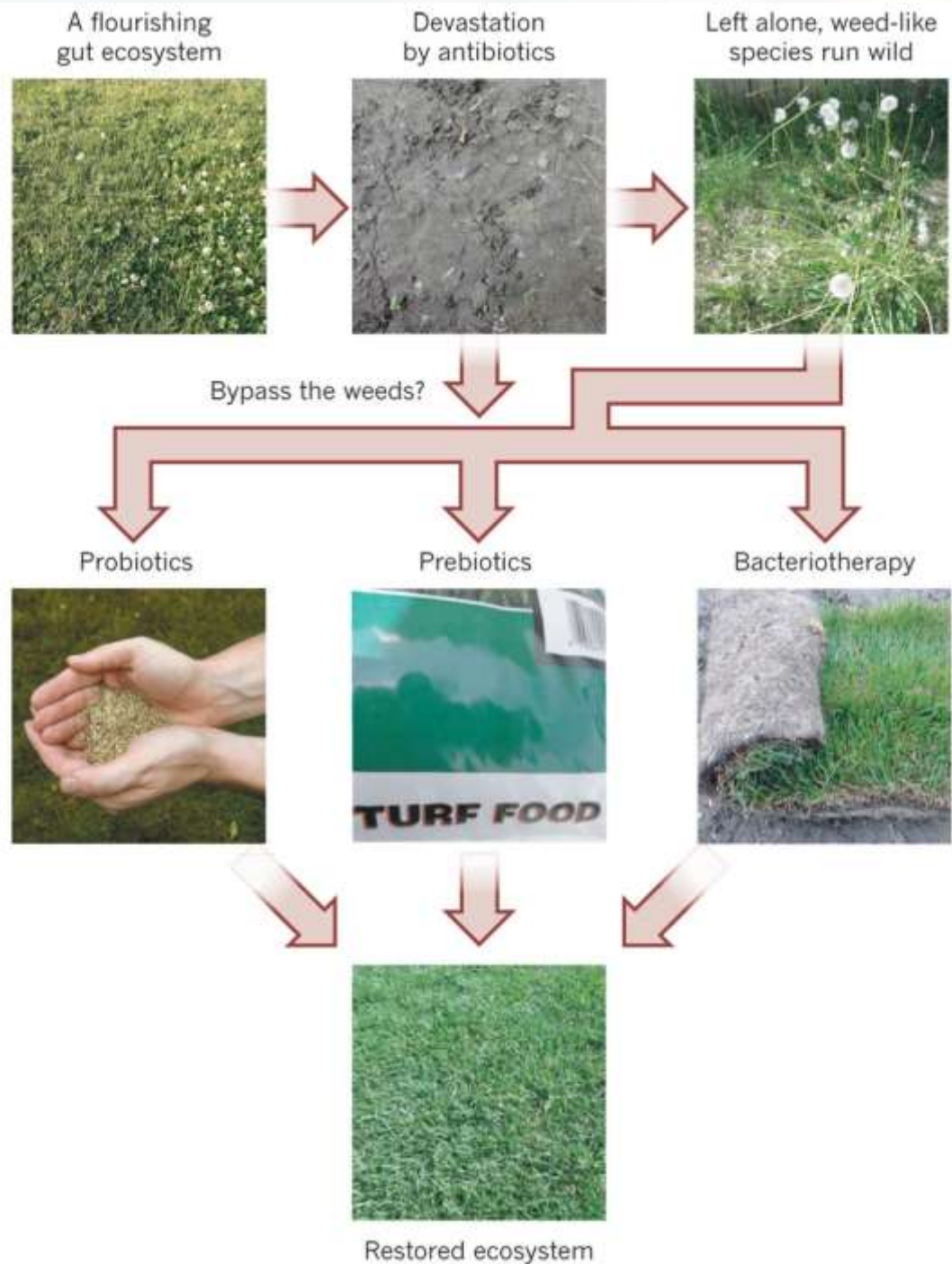


Allen et al. 2014 *Annals of the NY Acad Sci*



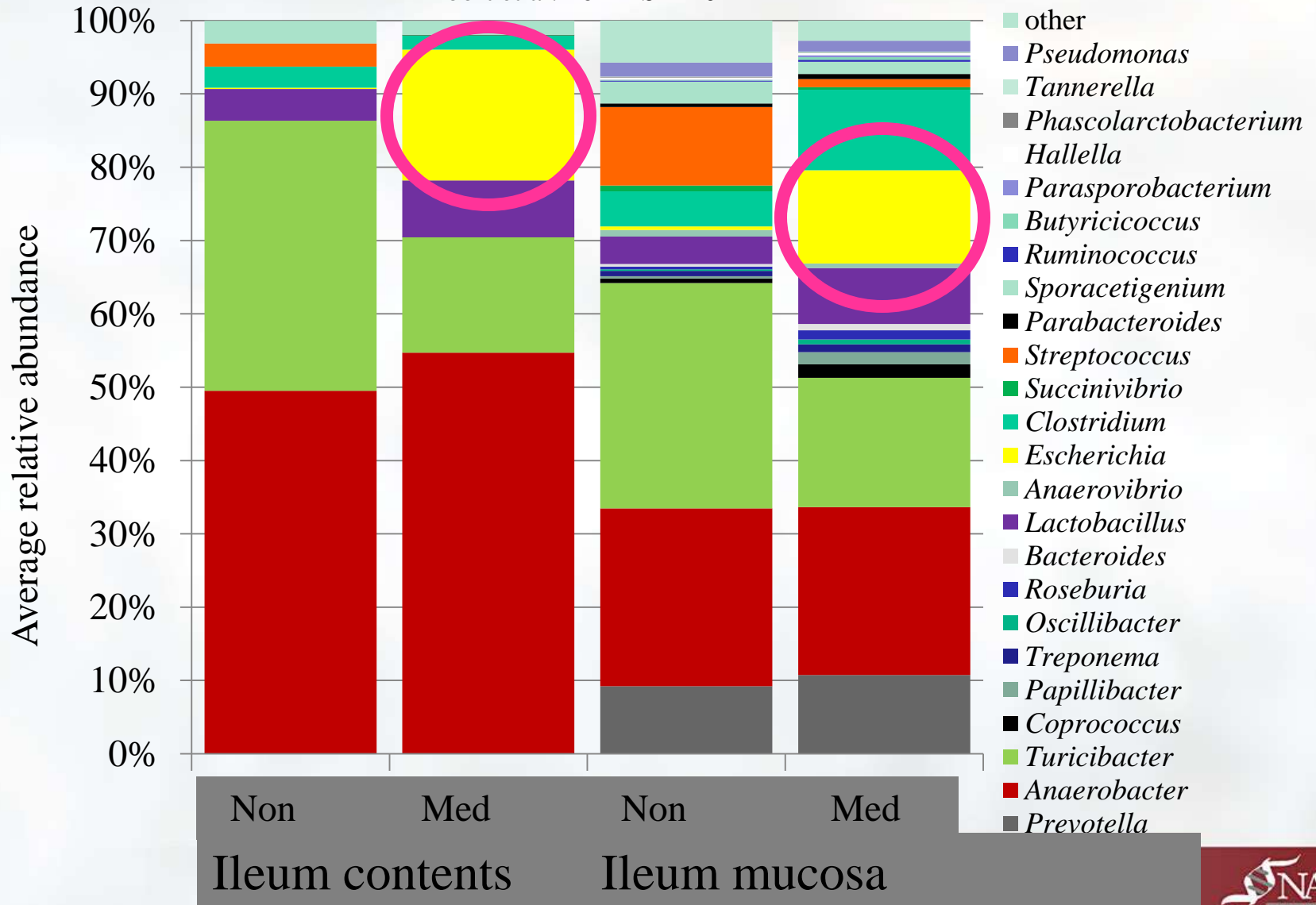
HOW?

- Disturbances
 - ◆ Antibiotics
 - ◆ Weaning
 - ◆ Transport
 - ◆ Infection
 - ◆ **STRESS**



E. coli populations increase with in-feed ASP250

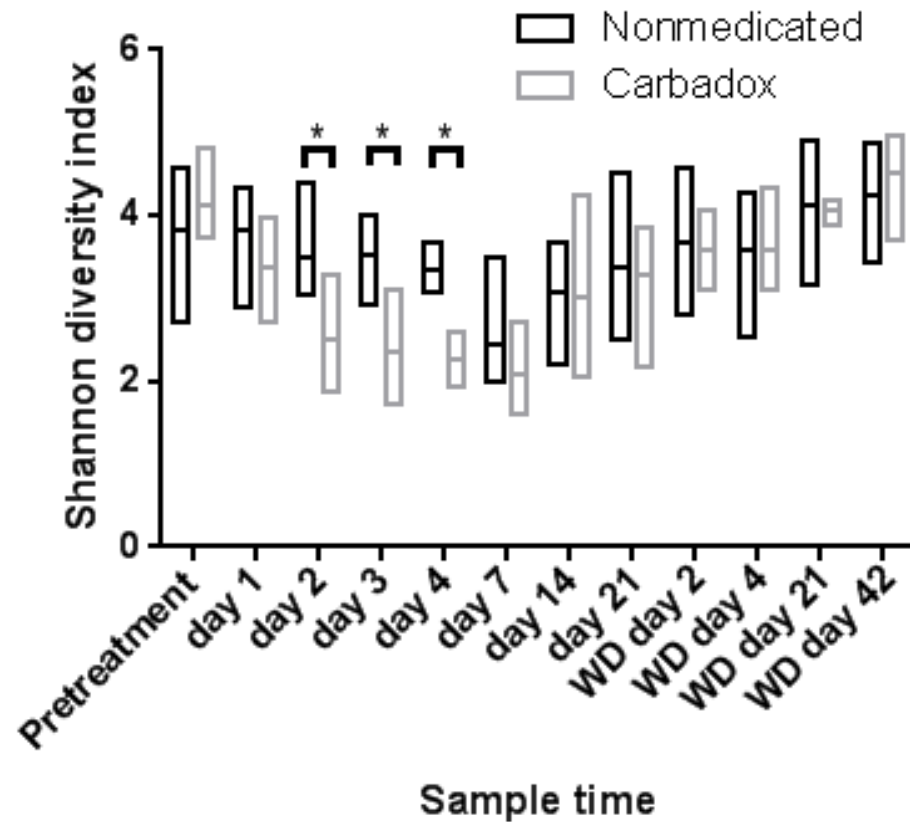
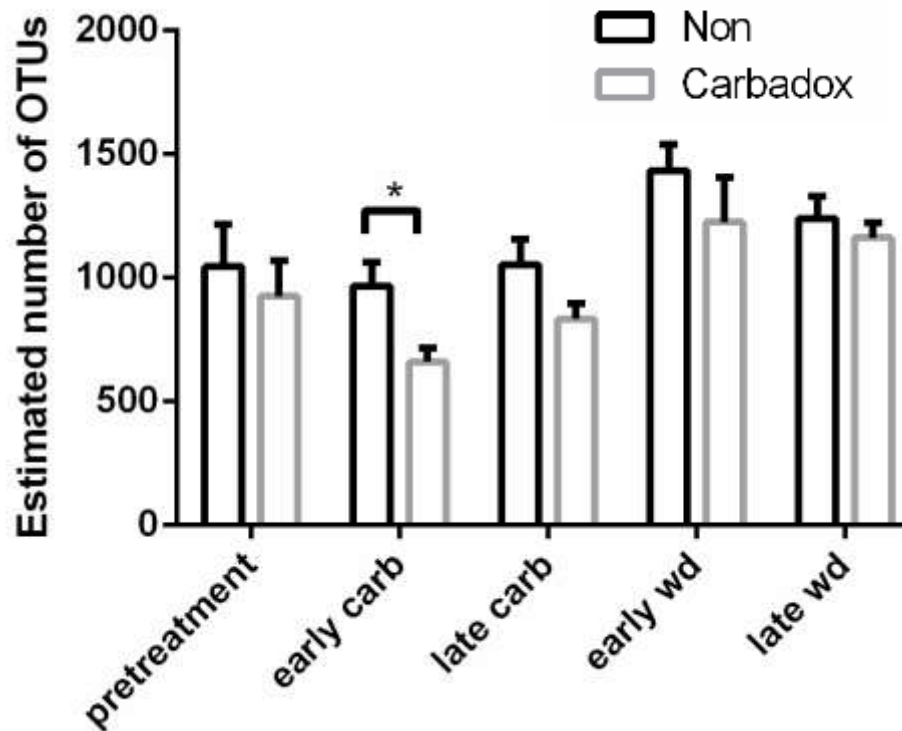
Looft et al. 2014 *ISME J*



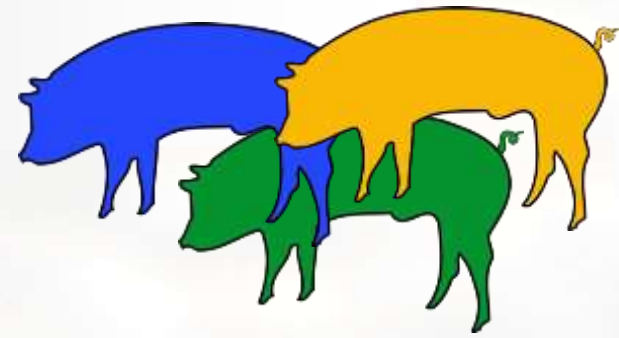
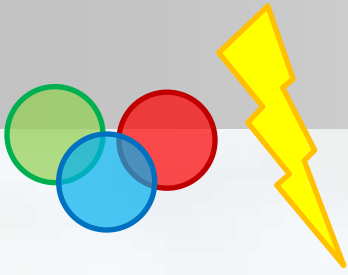
Microbiota Disturbance: Carbadox

- Important for the control of enteric diseases in swine at weaning, such as *Brachyspira hyodysenteriae*
- a quinoxaline-di-N-oxide antibiotic
- Mutagenic
- Fed to 1/3 of nursery age pigs in U. S.
- 42 day withdrawal period prior to slaughter
- It has no analog in humans; unclear if or how it will be regulated in the future

Carbadox causes a temporary decrease in gut bacterial diversity



Summary



- Not all disturbances are created equal.
 - ◆ Communities challenged with carbadox recovered in the presence of carbadox.
 - ◆ Communities challenged with *Salmonella* did not recover to pre-exposure composition.
- As yet, there is no “magic bullet” alternative to antibiotics
- Options include disease prevention via microbiota modulation

Future research

- Determine effects of other disturbances on gut microbiota (heat stress, feed additives)
- Gene expression analyses of gut bacterial communities
- Test novel beneficial bacteria of swine for probiotic efficacy

National Animal Disease Center USDA-ARS



54th Anniversary in 2015!



Thank you!

