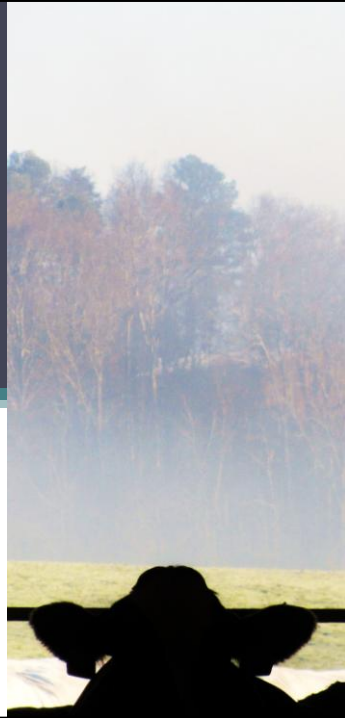


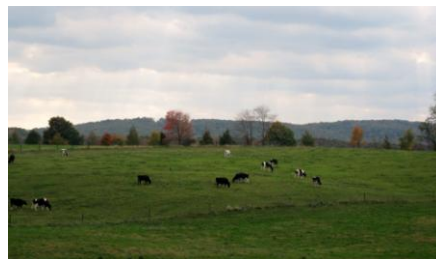
Antibiotic Resistance Genes and their Association in Dairy Cattle

Brittany Willing
Virginia Tech
February 23, 2013



Overview

- Antibiotic resistance genes (ARGs)
 - What are they?
 - Linked?
 - Multiple resistance?
- Reservoirs in the dairy industry
 - Ionophores?
 - Calves?
 - Cows?
- Reducing fecal ARGs



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Why Are ARGs Important?

- World Health Organization (WHO), called antibiotic resistance a critical human health challenge and expressed the need for “a global strategy to contain resistance”

(WHO Annual Report on Infectious Disease: Overcoming Antimicrobial Resistance)

- 2 million Americans infected, 14,000 die
- 75-95% of antibiotics can be excreted in an unaltered state

(Chee-Sanford *et al.*, 2009, Elmund *et al.* 1971, Feinman *et al.* 1978)

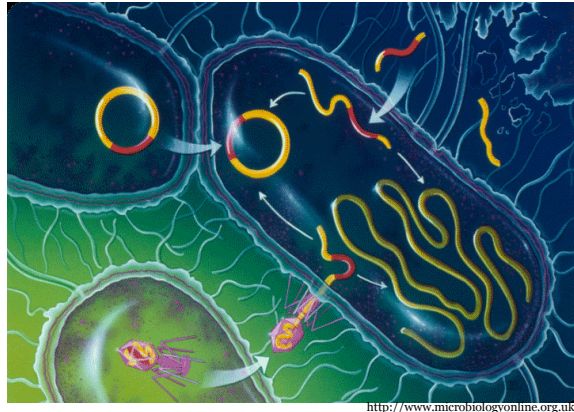
Why Are ARGs Important?

- Environmental Contaminant
- Animal Treatment Failure
- Human Treatment Failure
- Public Perception of the Dairy Industry



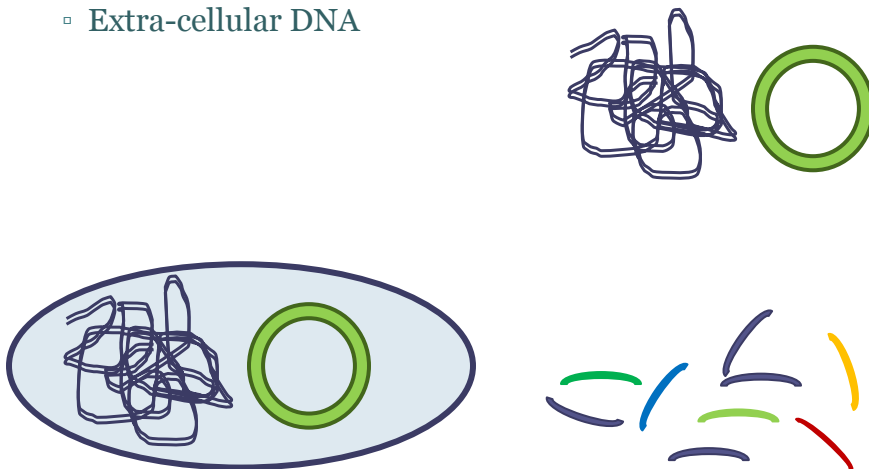
What are ARGs? Transference?

- Segments of DNA
 - mobile genetic elements
 - Plasmids
 - Transposons
 - Integrons
- Transformation
 - Extra-cellular DNA
- Conjugation
 - Horizontal gene transfer
- Transduction
 - Bacteriophage



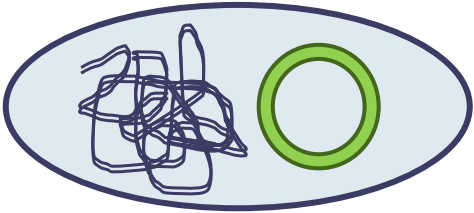
How are they transferred?

- Transformation
 - Extra-cellular DNA



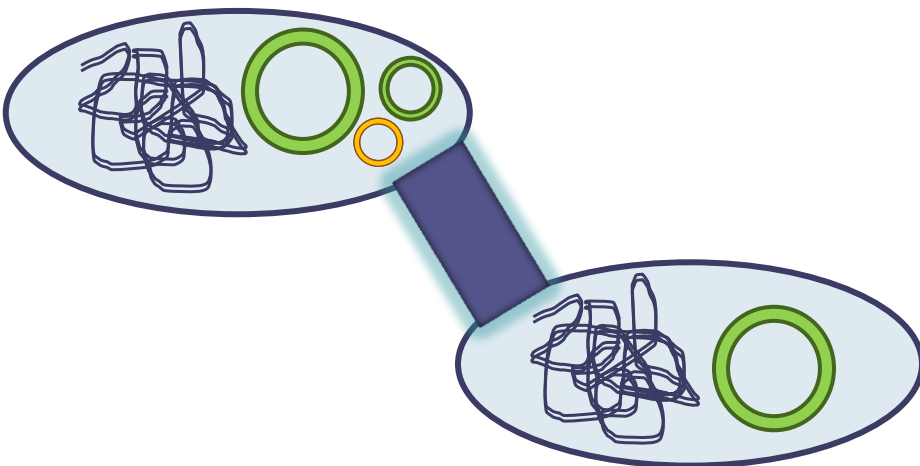
How are they transferred?

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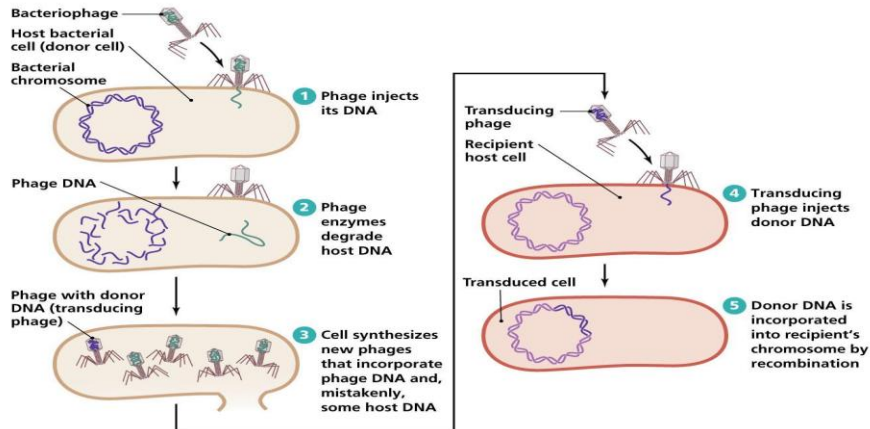
How are they transferred?

- Conjugation
 - Horizontal gene transfer



How are they transferred?

- Transduction
 - Bacteriophage



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How do ARGs spread so readily?

- Can ARGs be linked?
- Can bacteria carry multiple resistance?



Linked genes? Multiple resistance?

- Medicated milk replacer selected for resistance genes not used on-site (Berge *et al.* 2006)
 - Tetracycline hydrochloride, Neomycin sulfate
- Selected for:
 - Aminoglycosides (Neomycin)
 - Chloramphenicols (Chlor 500)
 - Sulfonamides (Albon)
- *E. coli* Isolates
 - 39% susceptible
 - 6% resistant to one antimicrobial
 - 55% multiply resistant

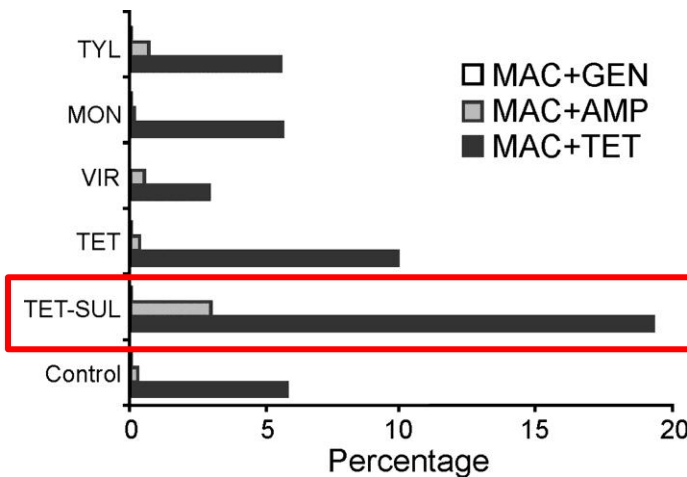


haccpeuropa.com

Berge *et al.* 2006

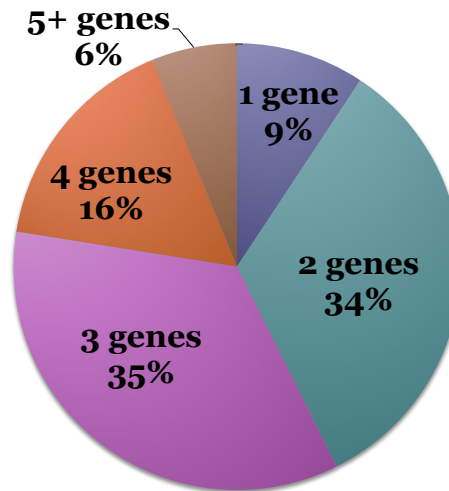
Linked genes? Multiple resistance?

- Beef feedlot cattle fed tetracycline + sulfamethazine

Alexander *et al.* 2008

Multiple Resistance From Mastitis Causing Bacteria

- 110 cows
- 135 *E. coli* isolates
- Panel of different antimicrobials



Srinivasan et al. 2007

Summary

- ARGs can be linked together
- High prevalence of multiple drug resistance
- Cattle are a reservoir for ARGs



<http://www.futurity.org>

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Where are the are the reservoirs?

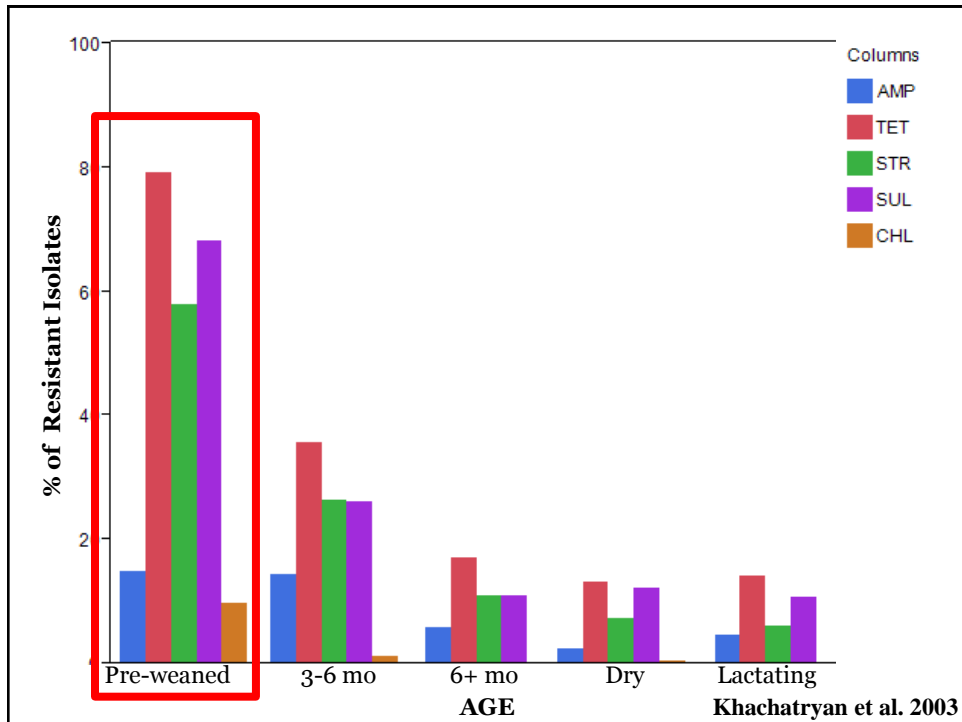


Ionophores

- Evidence of ionophore resistance in ruminants
 - 60% monensin (Dawson *et al.* 1983)
- Ionophore resistance is not readily spread between bacteria (Aarestrup *et al.* 1998, Aarestrup *et al.* 2000, Butaye *et al.* 2001)
- To date no ARG associated with ionophores have been elucidated nor do they have any effect on resistance in pathogens or their relative abundance

Where are the are the reservoirs?



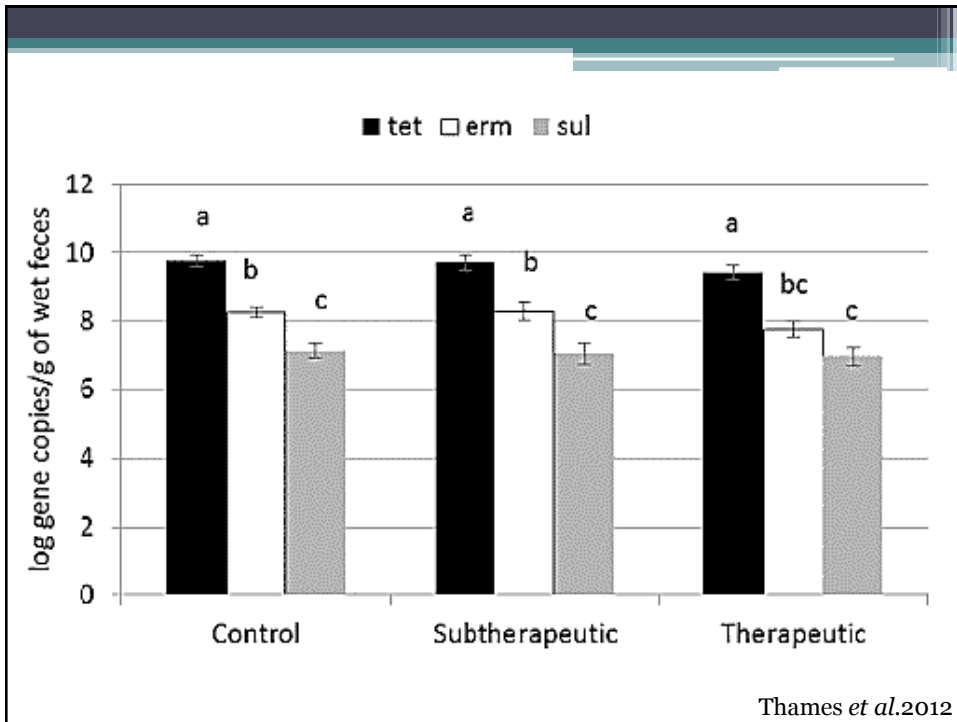


Calves: An Important Reservoir

- 1d
 - Control (No antibiotics)
 - Treatment 1 (Subtherapeutic, 10mg/calf/day)
 - Neomycin sulfate + oxytetracycline hydrochloride
 - Treatment 2 (Therapeutic, 1000mg/calf/day)
 - Neomycin sulfate + oxytetracycline hydrochloride



Thames et al.2012

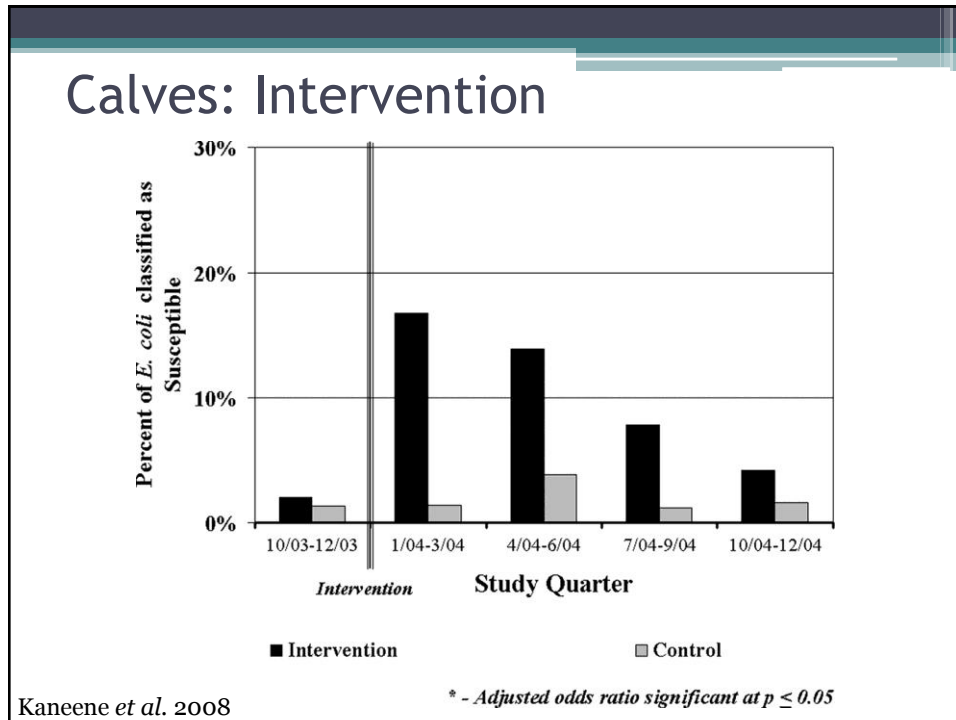


Calves: An Important Reservoir

- Conventional farms feeding oxytetracycline
- 8 farms
- 126 calves



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Summary

- Calves fed medicated milk replacer are a significant reservoir for antibiotic resistance genes
- Antibiotic resistance genes tend to decrease throughout life
- When treatment removed antibiotic susceptibility of bacteria decrease

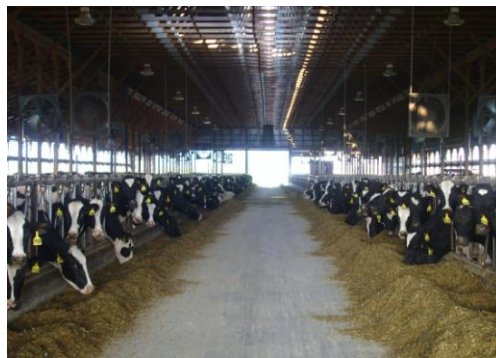


Where are the are the reservoirs?



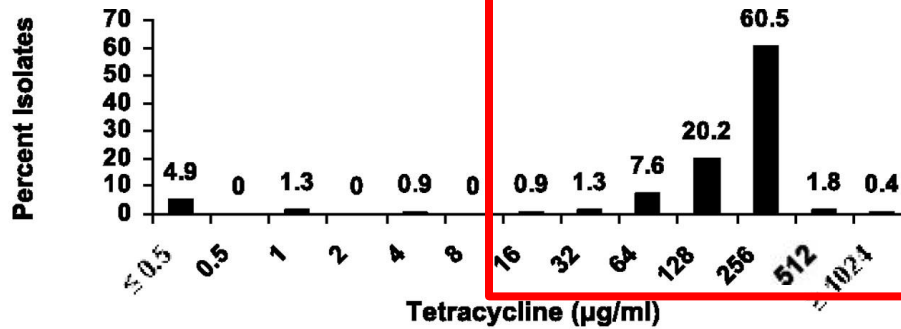
Lactating Dairy Cows

- 213 lactating cows
- 23 farms
- ~10% sampled from each herd
- Multiple resistance
 - 40.48%



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Lactating Dairy Cows



Resistance breakpoint: 16 µg/ml

Sewant *et al.* 2007

Where are the are the reservoirs?



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How Can Dairy Farmers Deal With These Issues?



Reducing Fecal ARGs

- Improve management practices in calves
 - Avoid using medicated milk replacers or waste milk containing antibiotics
- Manure management (Lactating herd)
 - Lagoons are a reservoir for ARG
 - Potential for degradation?



<http://walthorcountytoday.com>

Reducing ARGs in Lagoon

- Aerobic & Anaerobic
 - Ab Spiked
 - Killed
 - Ab Spiked and Killed
 - Background
- Antibiotics
 - Oxytetracycline
 - Sulfamethoxazole
 - Tylosin
 - Monensin



Pei *et al.* 2007

Reducing Lagoon ARGs

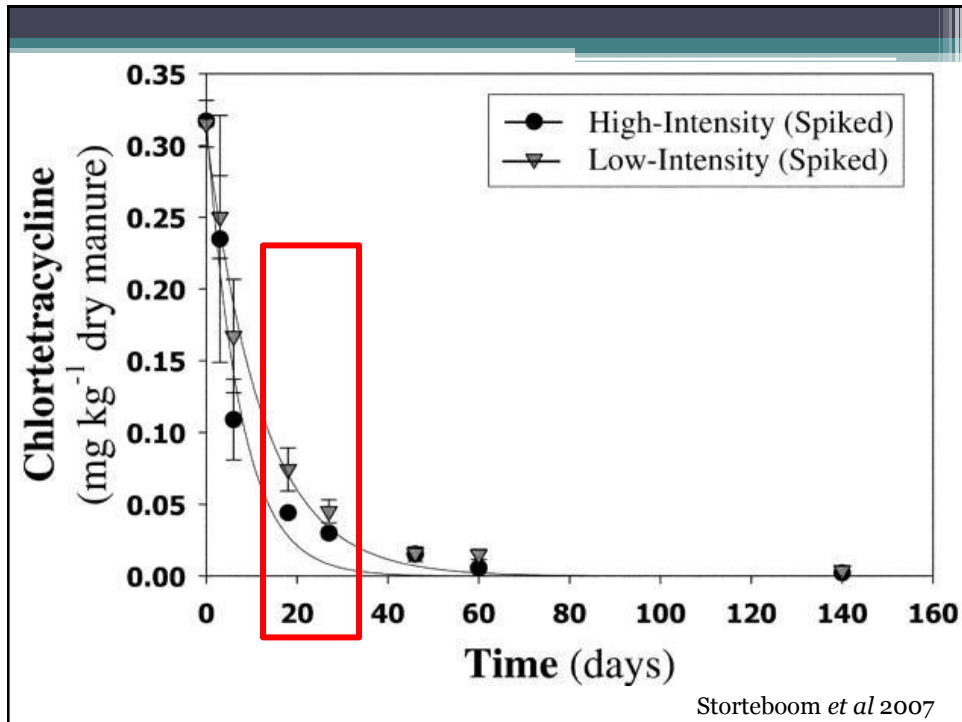
- Antibiotic degradation occurred in all 4 antibiotics tested
- Degradation occurred
 - Best at 20°C (68°F)
- Oxytetracycline degraded completely
 - Aerobic and Anaerobic
- Time is significant when degrading antibiotics and ARGs

Pei *et al.* 2007

Reducing ARG by Composting

- High Intensity
 - Amended with dried leaves and alfalfa
 - Watered as necessary
 - Turned weekly
- Low Intensity
 - No amendments, water, or turning





Degradation Summary

- Antibiotics can be degraded by various methods
- ARG, time is the most important factor



<http://www.2ropbooster.com/>

Take Home Message

- ARGs are an important public health concern
- Dairy cows and calves are important reservoirs for antibiotic resistance
- Antibiotic resistance genes are organic and natural and have the potential to be degraded



Questions?

