Effect of oxytetracycline on *Clostridium difficile* colonization and the fecal microbiome in veal calves

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CPHAZ Centre for Public Health and Zoonoses

University of Guelph — Changing Lives Improving Life

C. difficile in calves

- Animal health
  - Unclear role in enteric disease
- Public health
  - Concern about zoonotic/foodborne exposure
  - Common contamination of retail meat
  - Same strains in people and calves
    - Predominance of ribotype 078/TTV

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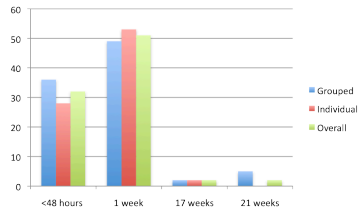

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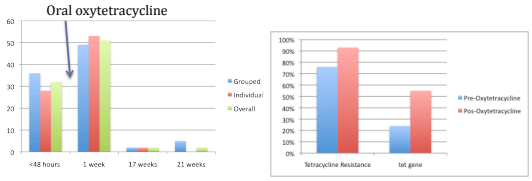
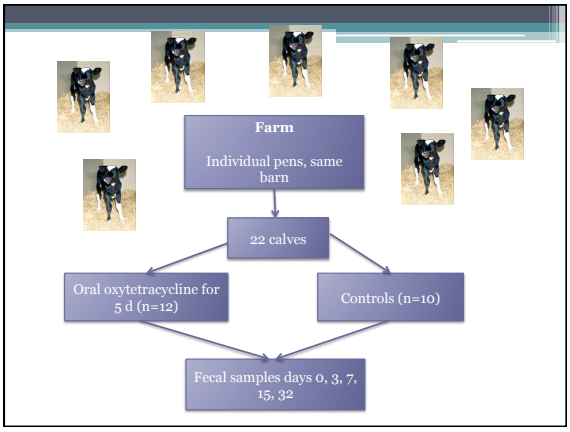
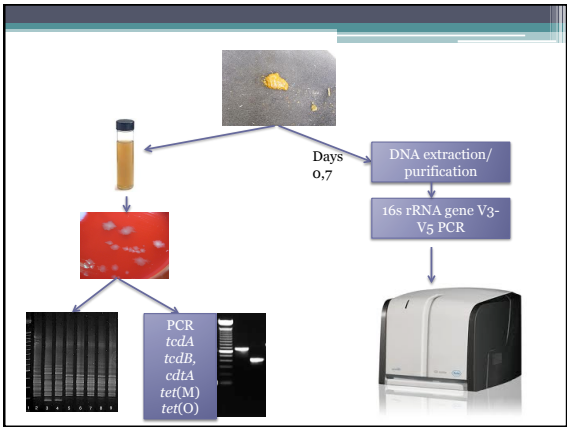


Ribotype	<48 h	1 wk	17 wks	21 wks	Overall
078	66% (37)	66% (58)	75% (3)	100% (4)	67% (102)
E	3.6% (2)	33% (29)	-	-	20% (31)
H75	7.1% (4)	-	-	-	3% (4)
Q	5.3% (3)	1% (1)	-	-	2% (4)
L	3.6% (2)	-	-	-	1% (2)
MOH AA	1.8% (1)	-	1 (25%)	-	1% (2)
OVC G	3.6% (2)	-	-	-	1% (2)
OVC AA	1.8% (1)	-	-	-	0.6% (1)
OVC J	1.8% (1)	-	-	-	0.6% (1)
F	1.8% (1)	-	-	-	0.6% (1)
R	1.8% (1)	-	-	-	0.6% (1)
CM 53	1.8% (1)	-	-	-	0.6% (1)
Total	37% (56)	58% (88)	2.5% (4)	2.5% (4)	100% (152)

- Does oxytetracycline therapy
  - Result in an increase in *C. difficile* shedding
  - Result in increased prevalence of tetracycline resistance in *C. difficile*
  - Result in a clonal shift of *C. difficile* strains, selecting for resistant strains
  - Alter development of the intestinal microbiome

Or…is this all an effect of normal aging or management??



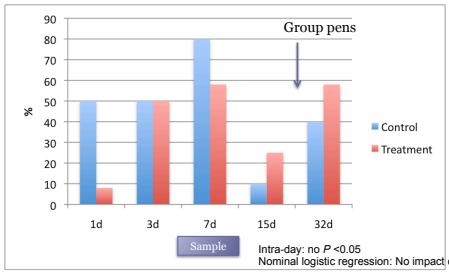


- MOTHUR
- Yue & Clayton coefficient: dissimilarity between groups
- Phylogenetic tree
  - similarity of populations among all samples
- Parsimony test
  - significance between groups
- Principal Coordinate Analysis (PCoA).
- Analysis of molecular variance (AMOVA)
  - compares distance between the PCoA center of each group.
- MG-RAST webserver (http://metagenomics.anl.gov)
  - Unpaired t-test for comparison of groups at different phylogenetic levels

Isolation

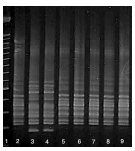
- *C. difficile* isolated from 47/110 (43%) samples
  - 24/60 (40%) treatment vs 23/50 (46%) controls (*P*=0.57)
- 21/22 (95%) positive on 1 or more samples
  - Treatment group: median 2, range 0-4
  - Control group: median 2, range 1-5
  - 7 calves had negative samples between positive samples
- Significant variability in prevalence between sampling days
  - Overall: *P*=0.006
  - Treatment group: *P*=0.04
  - Control group: *P*=0.04

Results



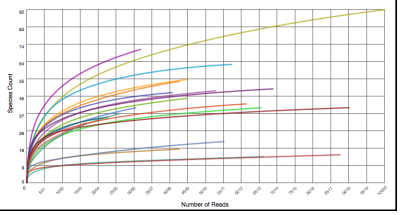
Characterization

- 89% toxigenic
  - Ribotype 078 (toxin A+,B+,CDT+): 94%
  - Ribotype F (A+, B+, CDT-, TT O): 6%
- Only ribotype 078 found after day2
- *tet*(M): 97%
- *tet*(O): 11%

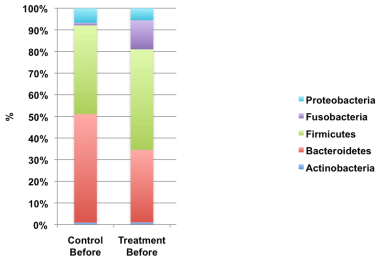


Fecal microbiome: days 0 and 7

- 119,909 high quality sequences post-QC
  - subsample of 1485 sequences/calf
- 747 unique OTUs
  - average: 46 OTUs/calf



Phyla



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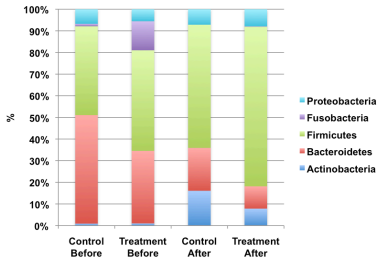
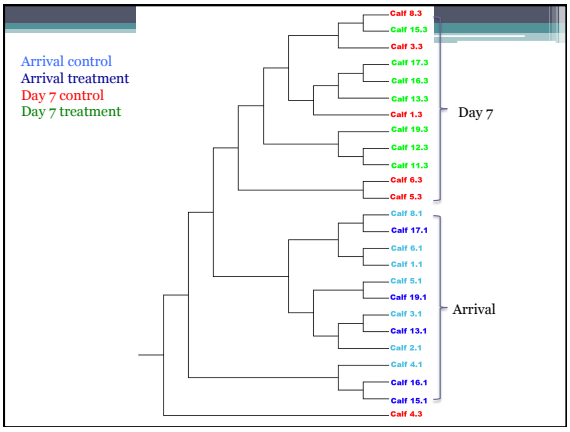


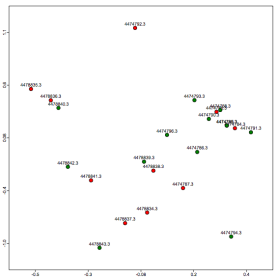
Impact of time

- Decrease in Bacteroidetes
  - Decrease in *Bacteroides* and *Prevotella* (P=0.0024 and P=0.0068)
- Increase in Actinobacteria
  - Increased Bifidobacteria (P<0.0001)
  - Decreased Coriobacteriales (P<0.0001)
- Increase in Firmicutes (P=0.0081)
  - Increase in Bacilli (P=0.0029).
  - Increase in *Lactobacillus* (P=0.0062)

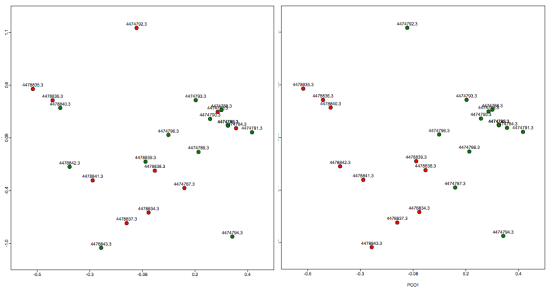


Group Comparison	Parsimony	AMOVA	t-test *
Control arrival x Control post-arrival	0.011*	0.001*	0.501
Treatment arrival x Treatment post-arrival	0.003*	0.013*	0.079
Control arrival x Treatment arrival	0.680	0.138	0.583
Control post-arrival x Treatment post-arrival	0.109	0.179	0.249
Control arrival x Treatment post-arrival	<0.001*	0.002*	0.044*
Treatment arrival x Control post-arrival	0.044*	0.016*	0.312

* Comparison of inverse Simpson's co-efficients



C. difficile positive/negative



C. difficile positive/negative

C. difficile Day 0/Day 7

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GUT MICROBIOTA ASSOCIATED WITH *Clostridium difficile* COLONIZATION IN INFANTS INVESTIGATED BY PYROSEQUENCING

Rousseau Clotilde[1,2], F. Levenez[3], J. Doré[3], A. Collignon[1,2], P. Lepage[3]

affected by *C. difficile* colonization. The kinetics analyse of the microbiota revealed that Ruminococcus gnavus (p = 0.007) was significantly associated with *C. difficile* colonization independently of the age of the infants. 16S rDNA sequences related to R. gnavus were more

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Calves:
R. gnavus: P=0.044
R. torques: P=0.046

Limitations

- Small sample size
- Single farm, single timeperiod
- Uneven baseline *C. difficile* prevalences

Discussion

- *C. difficile* is common in veal calves, with a predominance of ribotype 078
- *C. difficile* shedding and fecal microbiome change significantly after arrival at a veal farm
  - *Ageing and/or farm adaption appear to be more influential than antimicrobial administration*
- Multiple factors may be involved in fecal microbiome alterations
- Further study of the impact of environment, stress and management on the gut microbiome and *C. difficile* shedding in calves is required.

Antimicrobial administration certainly impacts shedding of some enteropathogens.

But maybe not always.
So do other factors.

Age and/or farm arrival stress may mask any effect of antimicrobials, at least initially.

Larger controlled studies of factors that influence the bovine gut microbiome are needed.

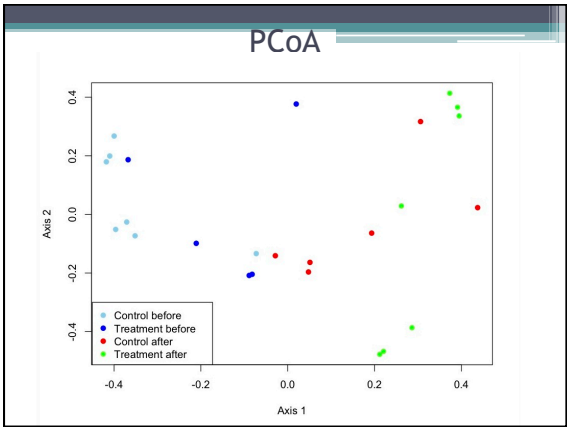
The simple answer isn't always the right answer.

The End

- Diversity: Simpson's inverse index of diversity

- Arrival: 3.3 ± 1.4
- After treatment: 2.4 ± 1.2 (P=0.14)

- Treatment: 2.9 ± 1.5
- Control: 2.7 ± 1.3 (P=0.67)



Study Population

- 22 Holstein bull calves (2-15 days of age)
  - Treatment group (n=12)
    - Oxytetracycline (6.6mg/kg PO, 5 days)
  - Control group (n=10)
    - No treatment
- Fecal samples
  - 24 hours, and days 3, 7, 15 and 32