

**The effect of antibiotic use on resistance in commensal *E. coli* from pre-weaned calves on a dairy farm in Washington State**

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## Antibiotic use in dairy calves

- Treatment protocols for observed syndromic diarrhea and respiratory cases
- Antibiotics are used to protect animal well-being (focus on preventing mortality)
- Management goal to use antibiotics carefully (focus on preventing mortality)

## Specific aims

- Document antibiotic use in pre-weaned calves
- Determine ABR patterns in commensal *E. coli* recovered from pre-weaned calves
- Evaluate impact of antibiotic use on ABR and analyze age and temporal trends in ABR patterns

## *E. coli* isolation & ABR testing

- Direct fecal culture on MAC agar
- 4 isolates picked per sample & *E. coli* confirmed using biochemical tests
- ABR testing using an agar micro-dilution assay with 13 antibiotics
- ABR determined as susceptible, intermediate and resistant & reclassified as susceptible and resistant

## Study design

- Repeated sampling within age cohorts (not necessarily the same animals) on a single farm
- Study conducted from June 2016 to January 2017
- Initial sampling interval 6 week, then 2 weeks thereafter
- Calves 1 – 11 weeks old
- Collected a total of 9 – 11 fecal samples from calves at each sampling
  - one randomly selected calf from each age category
  - all treatment history from a selected calf

## Data analysis

- Latent class analysis (LCA) of ABR data to create parsimonious (not perfect) groups to reflect resistance patterns to the tested antibiotics
- Latent regression analysis to determine associations of age and antibiotic treatment with resistance class

## 90% calves received antibiotic treatment

[treatment decisions are motivated by values and beliefs]

- 14 sampling dates, 140 fecal samples collected & 545 *E. coli* isolates characterized
- Treatment Frequency (60 day pre-weaning period)
  - 36/140-26% received a single antibiotic treatment**
  - 37/140-26% received 2 treatments
  - 30/140-21% received 3 treatments**
  - 23/140-16% 4-6 treatments
  - 14/140-10% no treatments
- Median age: 1x (9 days), 2x (15 days), 3x (25 days)

## 5 resistance classes

- LCA modeled 11 antibiotics (AN and AMC not used) & optimized at 5 resistance classes
  - 13% of the isolates had high probability of resistance to tetracycline -- **T**
  - 22% resistant to chloramphenicol (C), streptomycin (S), sulfisoxazole (Su) & T -- **CSSuT**
  - 23% extensively drug resistant (XDR) including nalidixic acid (Nal) resistance -- **XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup>**
  - 18% XDR including Xnl resistance -- **XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup>**
  - 24% XDR including Nal, Cip & Xnl resistance -- **XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup>**

## Fluoroquinolone resistance associated with enrofloxacin use but ceftiofur resistance not associated with ceftiofur use

- 45 calves (32%) treated with enrofloxacin
- E. coli* from enrofloxacin treated calves more likely to be resistant to ciprofloxacin (OR 2.5, 95% CI 1.7 – 3.7) and/or nalidixic acid (OR 2.8, 95% CI 1.9 – 4.0) than *E. coli* from calves not treated with enrofloxacin
  - (these also had greatest cumulative number of treatments)
- 3 calves (2.3%) treated with ceftiofur but 27% of *E. coli* resistant to ceftiofur

## Antibiotics used

[sequential trend following a treatment protocol – diarrhea to pneumonia]

- Sulfamethoxazole used most frequently (59%) for 1<sup>st</sup> treatment.
- Combination of ampicillin and sulfamethoxazole used (69%) for 2<sup>nd</sup> treatment.
- Enrofloxacin most commonly used for 3<sup>rd</sup> treatment.
- Enrofloxacin, florfenicol, or tulathromycin used if more than 3 treatments

## Latent regression analysis

- Association between age, treatment intensity and ABR class
  - 2 – 3 week olds more likely in XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup> class than 1 week olds (most likely to be in T class)
  - Calves that received 3 or 4-6 treatments more likely to be in XDR classes than untreated calves or calves that received 1 – 2 treatments

## Biological Summary and Conclusions

### Antibiotic Use and Intensity Matters

- 90% of calves in this study received an antibiotic at least one time and XDR was the prevalent ABR pattern
- Resistance class membership was age dependent
- Cumulative treatment intensity was associated with XDR
  - Enrofloxacin use was associated with fluoroquinolone resistance (NAL or CIP)
  - Ceftiofur resistance not associated with related antibiotic class use, i.e. ceftiofur

## Reflections on Biological Conclusions

- Off farm employees tasked with keeping calves healthy
- Role of veterinarian was to provide treatment protocols but use decisions are in the hands of employees
- Diagnostic and Treatment decisions are based on observation, experience, and belief with a focus to keep calves alive
- The progression of treatment decisions reflects progression of the farm treatment protocols
- The options available to employees for managing calf well being is not limited to antimicrobials but the belief in their efficacy to "heal" is unshakable

## 5 observed resistance classes

[XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup>, T, XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup>, CSSuT, XDR-Nal<sup>r</sup>Cip<sup>r</sup>Xnl<sup>r</sup> ]

