Antimicrobial susceptibility changes in *E. coli* from calves treated with chlortetracycline for anaplasmosis control

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Abstract

Background: In the U.S., chronic bovine anaplasmosis is commonly managed by protracted use of chlortetracycline (CTC)-medicated (0.5-2 mg/lb/BW/day) feed products, with no limit on duration of use. Prolonged antibiotic use may have unintended consequences including development of antimicrobial resistance in off-target microbes. The objective of this study was to evaluate changes in *Escherichia coli* antimicrobial susceptibilities from cattle provided CTC for chronic anaplasmosis control.

Materials and methods: Holstein-Jersey cross cattle with chronic anaplasmosis were blocked by weight, randomly allocated to one of the CTC treatment group (current FDA- approved dosages 0, 0.5, 2, and 10 mg/lb/BW/day) and fed their respective treatment for 120 days. *Escherichia coli* were isolated from fecal samples collected pre-treatment, after 58 and 114 days of consecutive treatment, and 21-days post-treatment cessation. Sensititre™ NARMS Gram Negative Plates were used to evaluate *E. coli* antimicrobial susceptibility to 14 antibiotics using CLSI breakpoints. The log-transformed minimum inhibitory concentration (MIC) data were subjected to linear mixed model analysis. Tests were performed at the 0.1 level with Tukey's multiplicity adjustment.

Results: The median MIC for tetracycline (TET) did not significantly change by treatment or over time. Median MICs for chloramphenicol, sulfisoxazole, trimethoprim/sulfamethoxazole, ampicillin, and streptomycin significantly increased within groups, sometimes crossing breakpoint classifications. Cefoxitin, azithromycin, gentamicin, nalidixic acid, and ceftiofur median MIC did not or minimally changed.

Conclusion: Under the conditions of this study, FDA-approved CTC dosages for active anaplasmosis control had minimal effect on increasing *E. coli* TET resistance, however, most isolates were already TET resistant. Increased *E. coli* resistance to other antibiotics did occur, however, indicating that long-term antibiotic use may broadly influence microbial antimicrobial susceptibility and highlighting the need for judicious antimicrobial use.

Introduction

- Bovine anaplasmosis is an economically important cattle disease with a global and continental United States presence.
- Clinical signs of anaplasmosis include anemia, reduced weight gain, decreased milk yield, infertility, and death in severe cases. Anaplasmosis is estimated to cost the U.S. cattle industry $300 million/year.
- Anaplasmosis is commonly controlled using chlortetracycline (CTC)-medicated feed products administered free choice (0.5-2.0 mg CTC/lb/BW/day) or hand-fed (0.5 mg CTC/lb/BW/day), without a limit on duration of use as long as the producer has a valid Veterinary Feed Directive.
- **Study rationale:** Antibiotic use can promote selection of resistant bacteria. Therefore, the protracted use of CTC for anaplasmosis control may promote selection of antimicrobial-resistance in off-target bovine-associated microbes.
- **Study objective:** Evaluate the change in antimicrobial susceptibilities of *Escherichia coli* isolated from fecal samples of cattle treated with different dosages of CTC for 120 consecutive days to a panel of 14 antibiotics
- **Study hypothesis:** Prolonged CTC treatment will increase recovery of tetracycline (TET) resistant *E. coli* with increased resistance occurring more quickly in cattle treated with greater CTC concentrations. In addition, promoting resistance to TET will co-select for resistance to other antibiotics as well.

Methods

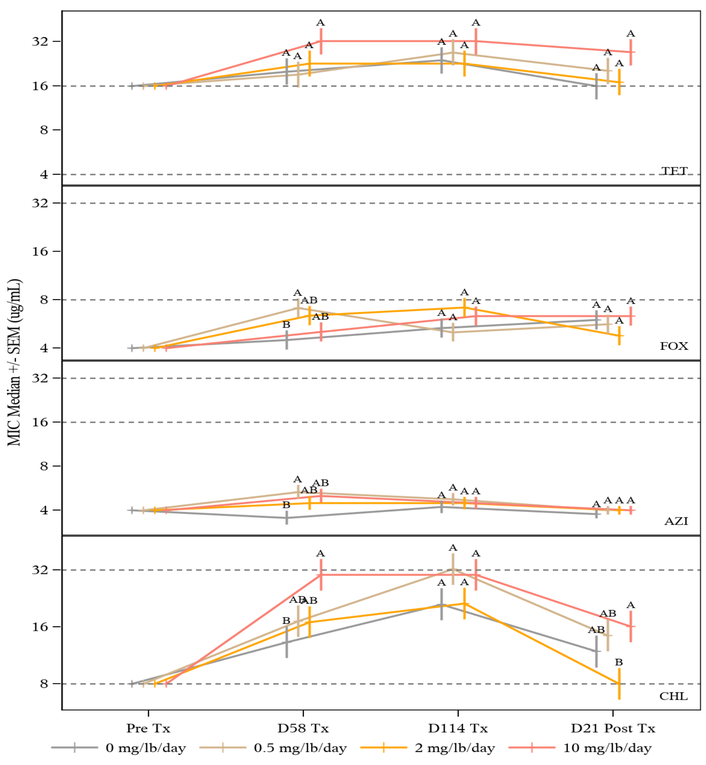
- Treatment groups: Holstein-Jersey cross cattle (~8-month-old steers) with chronic anaplasmosis were blocked by weight and randomly allocated to one of four CTC treatment groups (0-, 0.5-, 2-, and 10 mg/lb/BW/day).
- Treatment administration: The 0-, 0.5-, and 2-, mg/lb/BW/day groups were fed their respective treatment for 120 consecutive days while the 10mg/lb/BW/day group was administered treatment for 5 consecutive days (Mon-Fri) with 2 days off medicated-feed (Sat-Sun) during the 120-day treatment period.
- Sample collection: *Escherichia coli* were isolated from fecal samples pre-treatment, after 58 and 114 days of consecutive treatment, and 21 days post-treatment cessation.
- Determining *E. coli* antibiotic susceptibility: Sensititre™ NARMS Gram Negative Plates were used to evaluate *E. coli* antimicrobial susceptibility to 14 antibiotics using Clinical & Laboratory Standards Institute (CLSI) breakpoints. The log-transformed minimum inhibitory concentration (MIC) data were subjected to linear mixed model analysis.
- Statistical analysis: Log-transformed MIC data were subjected to linear mixed model analysis. Tests were performed at the 0.1 level with Tukey's multiplicity adjustment.

Results

Within-time point comparison of *E. coli* antibiotic susceptibility from isolates collected from different treatment groups.

The median *E. coli* MIC is presented for each treatment group at each time point. Within a time point, treatment groups with different letters are significantly different from each other.

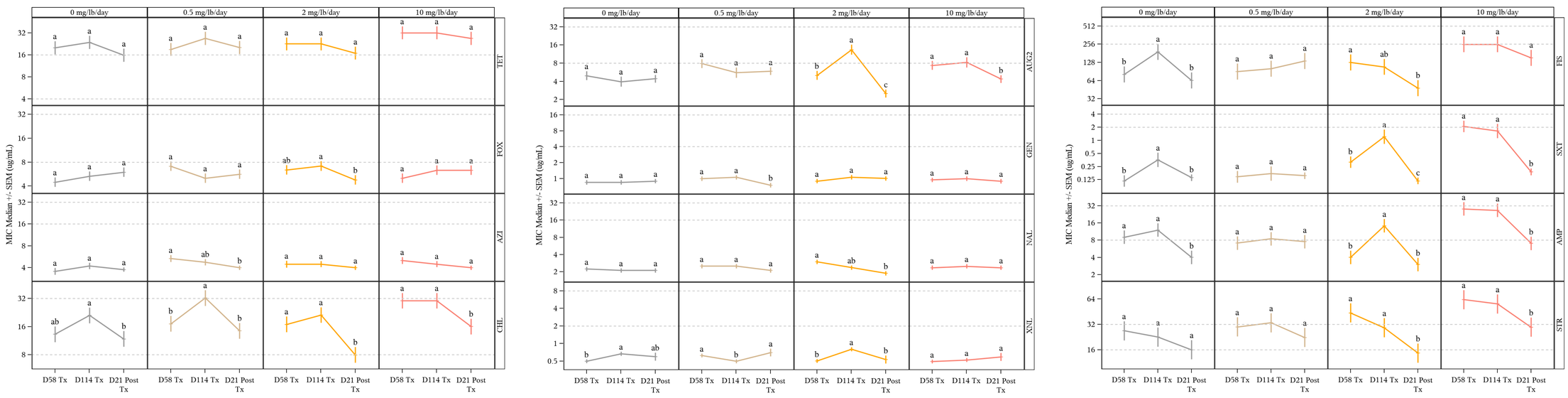
(Due to lack of variability, ceftriaxone and ciprofloxacin MICs were excluded from analysis.)



Across-time comparison of *E. coli* antibiotic susceptibility.

The median *E. coli* MIC is presented for each treatment group over time. Time points with different letter are significantly different from each other.

(Due to lack of variability, ceftriaxone and ciprofloxacin MICs were excluded from analysis.)



KEY: **Antibiotics: TET** (tetracycline), **FOX** (cefoxitin), **AZI** (azithromycin), **CHL** (chloramphenicol), **AUG2** (amoxicillin/clavulanic acid, 2:1 ratio), **GEN** (gentamicin), **NAL** (nalidixic acid), **XNL** (ceftiofur), **FIS** (sulfisoxazole), **SXT** (trimethoprim/sulfamethoxazole, 1:19 ratio), **AMP** (ampicillin), **STR** (streptomycin)

Dashed lines on graphs: The lower dashed gray line represent CLSI antibiotic susceptibility cut-off. The upper dashed gray line represents CLSI antibiotic resistance cut-off value. Only the MICs for the numerators were reported for AUG2 and SXT.

Discussion

- Under the conditions of this study, there was no change in *E. coli* resistance to tetracycline did not significantly change over the treatment period or between treatment groups. This is contrary to other studies which reported a direct selection of tetracycline-resistant *E. coli*. However, our findings may be explained as *E. coli* from all treatment groups were largely tetracycline resistant prior to treatment.
- A correlation between tetracycline, streptomycin, sulfisoxazole, ampicillin, ceftiofur, and chloramphenicol resistance has been reported. CTC treatment in this study resulted in a transient decrease in *E. coli* susceptibility to chloramphenicol , sulfisoxazole, ampicillin, and streptomycin; and MICs for these antibiotics had not returned to baseline median values by 21 days post-treatment cessation. The median MICs for chloramphenicol , sulfisoxazole, trimethoprim/sulfamethoxazole, and ampicillin, for 2 mg/lb and 10 mg/lb treatment groups almost crossed breakpoint classifications during the treatment period. The median MIC for streptomycin did cross the resistant breakpoint in the 10 mg/lb treatment group.
- This study provides evidence that prolonged use of CTC in cattle at doses approved to control active anaplasmosis imparts a selection pressure for resistance to multiple antibiotics in *E. coli*. CTC at 10 mg/lb/BW/day is not approved for the control of active anaplasmosis and was conducted strictly for research purposes only. However, a 5-day CTC treatment regimen at 10 mg/lb/BW/day is approved for the treatment of other infections. This study demonstrates that that increasing tetracycline concentration for anaplasmosis control may increase selection of multi-drug resistance *E. coli*.
- A limitation of this study was the sample size (number of cattle sampled, number of E. coli isolates evaluated from each steer) which could influence the magnitude of differences observed. Other variables that could also have influenced MIC results include ambient temperatures, layout of study site, etc.
- Future directions for this project include fecal sample metagenomics sequencing.

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