

Evaluation of the longitudinal effect of metaphylaxis treatment of preweaned dairy calves with enrofloxacin on the susceptibility of antimicrobial resistant fecal *E. coli*.

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Introduction

The use of antimicrobial drugs in food animals, specifically drugs in classes used also in human medicine, remains a contentious public health issue. Increasing concern with antimicrobial resistance has also enhanced the need for research that measure the impacts of utilizing antimicrobial drugs on antimicrobial resistance. The objective of this study was to longitudinally quantify *E. coli* resistant to ciprofloxacin and ceftiofur in calves treated with enrofloxacin or tulathromycin for control of bovine respiratory disease in high-risk calves.

Materials and Methods

Calves 2 to 3 weeks old were randomly selected and enrolled in each study group: (1) receiving single label dose of enrofloxacin (ENR) (Baytril 100, Bayer Corp. Agricultural Division, Shawnee Mission, KS); (2) receiving single label dose of tulathromycin (TUL) (Draxxin, Pfizer Animal Health); or (3) serving as a control and not receiving an antimicrobial treatment (CTL). Fecal samples were rectally collected from calves longitudinally starting just before the administration of the antimicrobial treatment and at days 2, 4, 7, 14, 21, 28, 56, and 112 days after beginning treatment, which will be referred from here on as time points. Collected samples were used for qualification of *E. coli* using a hydrophobic grid membrane filter (HGMP) master grid, which permits reliable replica plating of isolates and electronic enumeration. Enumeration of resistant *E. coli* was done by replicating these bacteria onto five individual agar plates to allow enumeration of susceptible and resistance isolates to ciprofloxacin and ceftriaxone.

E. coli counts were estimated using a formula that accounted for the number of dilutions used to reach ~50 to 150 isolated per grid, and other dilutions steps in processing the sample. Generalized linear mixed model (SAS, Institute, Cary, NC) was used, where the log₁₀ *E. coli* cfu/g of fecal sample was the dependent variable, treatment group and time points and interaction as independent variables, and animal identification and cohort test day as random effects. Effect of treatment group on the proportion of *E. coli* resis-

tant to ciprofloxacin and ceftriaxone was evaluated using Wilcoxon Sum Ranks test (JMP, Institute, Cary, NC) for each pair of treatment groups for each time point. A nonparametric test was used due to violation of normality assumption as determined using Shapiro-Wilk test. P values <0.05 were considered significant.

Results

No significant difference in the age of calves at enrollment for different treatment groups was observed. Treatment group did not have a significant effect on the cfu/g of *E. coli* over time (P value= 0.44). Regardless of treatment group, a significant gradual decrease in log₁₀ *E. coli* cfu/g was observed from time point 0 to 56 (Mean± SE 5.5± 0.05 to 5.0±0.05, respectively), and a significant peak in log₁₀ *E. coli* cfu/g at time point 112 (Mean± SE, 6.2±0.05) (P value <0.0001). Calves in the ENR treatment group has a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to CTL and TUL group at time points 2, 4 and 7. On time point 28, a significantly higher proportion of *E. coli* resistant to ciprofloxacin was observed only when compared to the CTL group. Calves in the TUL treatment group has a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to control group at time points 2, 4 and 7. None of the treatment groups resulted in significantly higher proportion of *E. coli* isolates resistant to ceftriaxone.

Significance

Our study identified that metaphylaxis treatment of calves with enrofloxacin resulted in significantly higher proportion of *E. coli* in fecal samples for up to 28 days after treatment. Metaphylaxis treatment of calves with tulathromycin resulted in significantly higher proportion of *E. coli* in fecal samples for up to 7 days after treatment. These findings highlight the importance of cautious selection and use of antimicrobials for metaphylaxis.