

<b>Project Title:</b>	Effects of Chlortetracycline and Ceftiofur Treatment on Prevalence, Quantity and Antimicrobial Resistance of <i>Salmonella</i> in Feedlot Cattle
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### Background

The threat of antimicrobial resistant bacteria and the implications for human health is an area of research that has been receiving a great deal of attention in both the media and scientific community. In the Centers for Disease Control and Prevention report, "Antibiotic Resistance Threats in the United States, 2013," *Salmonella* is listed as a microorganism of serious threat. *Salmonella* outbreaks are most commonly associated with poultry and eggs; however, beef has also been implicated as a source of infection. The use of antibiotics in food animals may contribute to dissemination of antimicrobial resistant bacteria through the food chain. One of the major concerns is that many antibiotics used in food animals are also important in human medicine. Strict regulations or bans on the use of antimicrobials in agriculture could have devastating effects on animal health and create food safety issues.

The objective of this study was to determine the effects of chlortetracycline and ceftiofur treatment on the prevalence, quantity and antimicrobial resistance of *Salmonella* in feedlot cattle.

### Methodology

Individual fecal samples were collected at an experimental feedlot at West Texas A&M University in Canyon, Texas. The randomized and controlled field trial was comprised of 176 steers in four different treatment groups to assess the impact of ceftiofur and chlortetracycline use. Sixteen pens (11 steers/pen) were subjected to 4 treatment regimens; 1) all 11 steers in 8 pens were treated with ceftiofur on day 0; 2) 4 of these pens followed with intermittent chlortetracycline from day 4 through day 19; 3) 1 out of 11 steers in the remaining 8 pens were treated with ceftiofur on day 0; 4) 4 of these pens received chlortetracycline as above. Cattle fecal samples from days 0, 4, 14 and 26 were enriched and plated to selective media to identify *Salmonella* positive samples. Spiral plating onto brilliant green agar was used to obtain crude estimates of *Salmonella* quantities. Antibiotic susceptibility was performed for 11 different antimicrobials by the micro-broth dilution method using the Sensititre<sup>®</sup> automated inoculation delivery and fluorometric plate reading system. Genotypic antimicrobial susceptibility profiles were determined by whole genome sequencing of *Salmonella* isolates. Sequencing data was analyzed for the presence of antimicrobial resistance genes using the online databases Resfinder and CARD. *Salmonella* serotypes were determined using whole genome sequencing data and short read sequence typing for bacterial pathogens. Two samples of each serotype determined by SRST2 were sent to National Veterinary Services Laboratory for confirmation using standard serotyping methods.



## Findings

The prevalence of *Salmonella* in cattle fecal samples was higher than researchers anticipated. The high prevalence of *Salmonella* was most likely due to geographic clustering and climate at the time of sampling. Treatment with ceftiofur and chlortetracycline showed a decrease in the prevalence of *Salmonella* in cattle fecal samples. The prevalence of *Salmonella* positive fecal samples declined from a baseline value on day 0 at 87% to 38.6% on day 4 in cattle treated with ceftiofur. On day 14, the prevalence of *Salmonella* positive fecal samples had started to increase in cattle only treated with only ceftiofur; however, the prevalence for cattle additionally treated with chlortetracycline declined to 34%. The fecal sample prevalence of cattle that had not been treated with ceftiofur but received chlortetracycline was also 34%. This suggests that chlortetracycline alone was as effective as treatment with both ceftiofur and chlortetracycline in reducing the fecal prevalence of *Salmonella* in cattle. On day 26, the fecal prevalence in all four treatment groups had increased once again and was approaching baseline values from day 0.

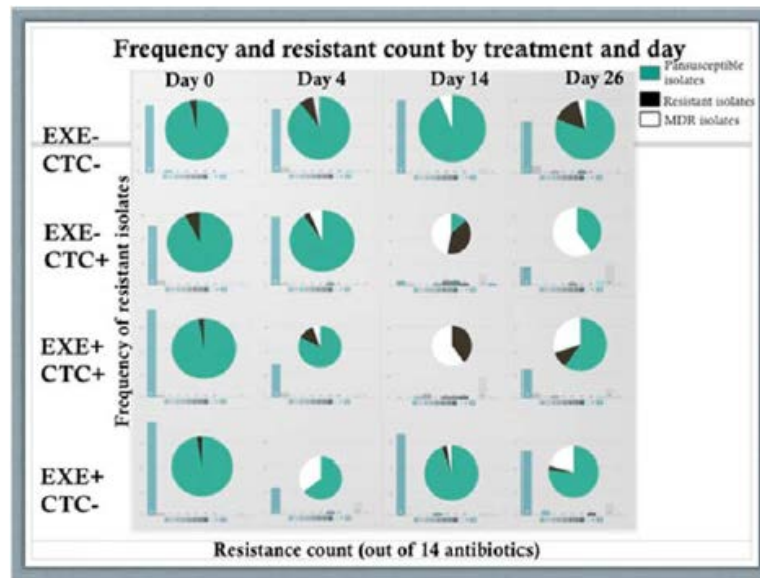
Although ceftiofur and chlortetracycline treatments were effective in reducing the fecal prevalence of *Salmonella* in cattle, the remaining *Salmonella* population after treatment was much different than the baseline population. On day 0, the majority of the present *Salmonella* was pan-susceptible. On day 4 and 14, researchers saw an increase in the proportion of resistant and multidrug-resistant *Salmonella* in the treatment groups that had received ceftiofur and/or chlortetracycline. The *Salmonella* population on day 26 was comprised of both pan-susceptible and multidrug-resistant isolates rather than the predominantly pan-susceptible population observed on day 0. The majority of the multidrug-resistant *Salmonella* isolates were serotype Reading and resistant to 9 different antibiotics. These multidrug-resistant isolates showed a correlation between phenotype and genotype and harbored *tetA*, *bla<sub>cm</sub>y2-like*, *sull*, *sulII*, *strA*, *strB* and *floR* genes.

## Implications

The dynamics of the *Salmonella* population in cattle at slaughter has important food safety implications. This study showed that on day 26 following administration of ceftiofur and/or chlortetracycline, an increase in the proportion of antibiotic resistant *Salmonella* was seen. If this population of antibiotic resistant *Salmonella* remains stable or increases as the cattle progress to slaughter, there is the potential for the dissemination of these resistant bacteria through the food chain. Further studies are needed to determine if and how the *Salmonella* population changes from day 26 to slaughter following the administration of antibiotics.

**Table 1.** Prevalence of *Salmonella* positive fecal samples across treatment groups and sampling days. Treatment groups in which only one steer in the pen received ceftiofur are labeled EXE- and treatment groups in which all steers in the pen received ceftiofur are labeled EXE+.

Treatment Group	Day 0 #positive (%)	Day 4 #positive (%)	Day 14 #positive (%)	Day 26 #positive (%)
EXE+/CTC-	39/44 (88.6)	17/44 (38.6)	35/44 (79.5)	34/44 (77.3)
EXE+/CTC+	38/44 (86.4)	17/44 (38.6)	15/44 (34.1)	20/44 (45.5)
EXE-/CTC-	29/44 (65.9)	29/44 (65.9)	32/44 (72.7)	26/44 (59.1)
EXE-/CTC+	27/44 (61.4)	32/44 (72.7)	15/44 (34.1)	20/44 (45.5)



**Figure 1.** Count of *Salmonella* isolates resistant to the different number of antibiotics and frequency of pan-susceptible, resistant, and multidrug-resistant isolates. The bar charts represent the counts and the pie charts represent the frequency.