Efficacy of ceftiofur for treatment of experimental salmonellosis in neonatal calves

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Objective—To evaluate therapeutic efficacy of a high extralabel dose of ceftiofur for treatment of experimental salmonellosis in neonatal calves.

Animals—Forty-two 1- to 4-day-old Holstein bull calves.

Procedure—36 calves were orally challenged with *Salmonella enteritica* serovar Typhimurium (6.5 × 10⁶ colony-forming units). Six additional calves were retained as nonmedicated nonchallenged control calves. Four days following *Salmonella* challenge, surviving calves were randomly allocated to ceftiofur-treated (5 mg/kg, IM, q 24 h) or nonmedicated control groups. Calves assigned to the treated group were medicated daily for 5 days starting on day 4 after challenge. Calves were monitored for 18 days following *Salmonella* challenge. Outcome assessments included clinical parameters (attitude, appetite, fecal characteristics, and rectal temperature), mortality rate, and quantitative *Salmonella* culture of fecal samples, mesenteric lymph nodes, and cecal contents.

Results—Ceftiofur treatment was associated with a significant decrease in rectal temperature and diarrhea. Three of 15 medicated calves and 4 of 14 nonmedicated calves died or were euthanatized between days 4 and 18. A significant decrease in fecal shedding of *Salmonella* organisms was observed in treated calves, compared with nonmedicated calves. *Salmonella* organisms were isolated from all 10 nonmedicated calves at necropsy, whereas no *Salmonella* organisms were isolated from 5 of 12 medicated calves.

Conclusions and Clinical Relevance—Treatment of salmonellosis in neonatal calves with a high extralabel dose of ceftiofur (5 mg/kg, IM, q 24 h) promotes animal welfare, reduces fecal shedding of *Salmonella* organisms, and may promote clearance of *Salmonella* infections when plasma ceftiofur concentrations are maintained above minimal inhibitory concentrations.

While most *Salmonella* infections in cattle are subclinical, outbreaks of clinical disease frequently occur in postpartum cows and in neonates during the first 4 weeks of life. *Salmonella enterica* serovar Typhimurium is the most common *Salmonella* serotype associated with clinical disease in calves. High morbidity and mortality rates are a common feature of neonatal disease outbreaks with this serotype. Veterinarians presented with an outbreak of salmonellosis in calves need to identify critical control points and implement appropriate management interventions to prevent disease. Implementation of a therapeutic plan is necessary to alleviate animal suffering and loss. Appropriate management of sick calves is also important to avoid amplification of environmental contamination and the risk of disease transmission. Dehydration, electrolyte imbalances, endotoxemia, and bacteremia are common features of salmonellosis in neonatal calves. Treatment is directed at replacing fluid and electrolyte losses, limiting inflammatory cascades to prevent septic shock through use of nonsteroidal anti-inflammatory drugs, and controlling bacteremia through judicious use of antimicrobial drugs.

Currently, there is a void of antimicrobials labeled for treatment of salmonellosis in cattle. Consequently, extralabel antimicrobial use for treatment of salmonellosis frequently occurs in veterinary practice in the United States. Several antimicrobial efficacy studies performed 15 to 25 years ago evaluated therapeutic antimicrobial use for treatment of salmonellosis in neonatal calves. Antimicrobials evaluated in these experimental therapeutic trials included amoxicillin, ampicillin, trimethoprim-sulfadiazine, and chloramphenicol. In these experimental clinical trials in which the *Salmonella* challenge strain was susceptible to the therapeutic antimicrobial agent tested, treatment decreased the severity of clinical disease, and a decrease in fecal shedding of *Salmonella* organisms was reported in the 1 study in which it was quantified. Use of chloramphenicol to treat food animals is illegal in most countries, and antimicrobial resistance commonly limits the efficacy of ampicillin, amoxicillin, and trimethoprim-sulfadiazine against *Salmonella* infections.

Efficacy data for newer-generation antimicrobials are needed to facilitate rational therapeutic antimicrobial selection and to accurately weigh the benefits and risks associated with antimicrobial drug use in food animals. The objectives of the current study were to evaluate the therapeutic efficacy of ceftiofur, used at a high extralabel dose, for treatment of salmonellosis in neonatal calves and to determine the effect of such ceftiofur treatment on fecal shedding of *Salmonella* organisms in an experimental neonatal calf challenge model.

Materials and Methods

Experimental animals—The study was conducted under a protocol approved by the University of California, School of Veterinary Medicine, Department of Medicine and Epidemiology (House, Tankersley, Ontiveros, Alcantar, Smith), School of Veterinary Medicine, University of California, Davis, CA 95616; and Pharmacia Animal Health, 7000 Portage Rd, Kalamazoo, MI 49001 (Kotarski). Dr. House’s current address is The University Veterinary Centre, University of Sydney, New South Wales, Australia. Funded by Pharmacia Animal Health, Kalamazoo, Mich.

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Davis, Animal Use and Care Committee. Forty-two newborn Holstein bull calves (body weight, 32 to 59 kg) were obtained from a single dairy during a 96-hour period. Calves received 2 feedings of a colostrum supplement via an esophageal feeder within 9 hours of birth (90 g of IgG). A third feeding was offered via a bottle 8 hours following the second feeding. Calves refusing the third feeding at 8 hours were fed 12 hours following the second feeding. Calves received a total of 135 g of IgG during the first 24 hours of life. Following administration of colostrum, calves were weighed and transported to the Animal Resources Facility at the University of California, Davis. Calves were housed in triplex hutches with solid partitions between calves. Blood samples were collected from each calf 24 hours following the last colostrum feeding for determination of total serum protein concentration. Calves were fed a nonmedicated milk replacer that contained 20% fat and 20% protein for the duration of the study (1.89 L [2 qt] twice a day for days 0 through 10 and 2.36 L [2.5 qt] twice a day for days 11 through 18). The first 36 calves born were assigned to the 5 Typhimurium challenge group, and the last 6 calves were kept to serve as nonchallenged control calves. The random allocation of calves to the challenge group was to minimize the age distribution of the challenge group. Salmonella-challenged calves were housed in a separate facility from nonchallenged control calves to avoid cross contamination between groups. Three days following challenge, surviving calves (n = 29) were randomly allocated into medicated (15) and nonmedicated (14) groups. On study day 18, all remaining calves were weighed and euthanized via IV administration of pentobarbital sodium.

**Challenge procedure**—When the youngest calf in the challenge group was 24 hours old (range, 24 to 108 hours old), all challenge calves received 6.5 X 10^5 colony-forming units (CFU) of S Typhimurium (TY1212) orally. Food was withheld from calves for 10 hours prior to challenge, and calves were fed milk replacer 30 minutes following challenge. The minimal inhibitory concentration (MIC) of ceftiofur for the challenge S Typhimurium strain (TY1212) was 1 µg/mL. The S Typhimurium strain (TY1212) was isolated from an outbreak of salmonellosis on a calf ranch in southern California and found to be resistant to florfenicol, kanamycin, spectinomycin, and tetracycline. The challenge was prepared by inoculating 1 L of Luria Broth with a single colony of S Typhimurium (TY1212). The broth was incubated at 37°C for 18 hours, yielding an estimated final cell concentration of 5 X 10^7 CFU/mL. Cells were harvested by centrifugation at 4°C and the cell pellet suspended in 0.2M Na2HPO4. Each 10-ML challenge dose was drawn into a sterile disposable syringe to give a final dose of 6.5 X 10^6 CFU of S Typhimurium (TY1212). Prior to and during challenge, each challenge dose was kept on ice. Following challenge, serial 1:10 dilutions of an extra challenge dose were plated and colonies counted to verify the dose administered.

**Ceftiofur treatment**—Four days following oral challenge, surviving calves were randomly assigned to medicated and nonmedicated groups by use of random case selection software.7 Calves in the medicated group (n = 15) received ceftiofur sodium7 (5 mg/kg) via IM injection once a day following the morning feeding and assessment of clinical parameters. For efficacy, serum concentrations of β-lactam antimicrobials should be continuously above the MIC for target organisms.7 Higher doses of ceftiofur have been used in experimental trials without adverse effects. Calves were medicated for 5 days starting on day 4 (86 hours following challenge). Initiation of treatment was timed to follow the onset of clinical disease, as determined in a prior challenge model experiment.

**Therapeutic drug monitoring**—Blood samples were collected from all medicated calves 2, 6, 12, and 24 hours following treatment on study days 4, 6, and 8 to measure plasma ceftiofur and ceftiofur metabolite concentrations. Prior to analysis, samples were stored at −3°C. All plasma samples were analyzed following the approved high-performance liquid chromatography method for determination of ceftiofur residues in bovine and swine plasma.8 This assay converts all ceftiofur and desfuroylceftiofur (DFC) conjugates to a single derivative, DFC acetamide. Briefly, ceftiofur and all DFC metabolites were reduced by use of diithioerythritol to free DFC, which was then stabilized with iodoacetamide. These reactions were performed on-column by use of solid phase extraction technology. Desfuroylceftiofur acetamide was then quantified by high-performance liquid chromatography by use of a C18 reverse-phase analytical column with ultraviolet detection at 255 nm. Recoveries are 90 to 100% with a mean coefficient of variation of 10%.8 We have found that ceftiofur added as an internal standard is stable to 2 freeze-thaw cycles at −15°C. The limit of quantification was 0.150 µg of ceftiofur equivalents/mL of plasma. Only values greater or equal to the limit of quantification were reported.

**Clinical assessment**—Clinical parameters were recorded each morning. Parameters evaluated included attitude, appetite, fecal characteristics, and rectal temperature. Attitude and appetite were scored with an ordinal scale. Attitude was scored on a scale of 0 to 4 as follows: 0 = standing to be fed, 1 = stands with stimulus, 2 = stands with assistance, 3 = maintains sternal recumbency, and 4 = lateral recumbency. An attitude score of 4 constituted grounds for euthanasia at any time during the day. Appetite was scored on a scale of 0 to 3 as follows: 0 = consumed ≥ 1.89 L, 1 = consumed < 1.89 L but > 0.945 L, 2 = consumed < 0.945 L, and 3 = no appetite. Appetite score was also recorded for the afternoon feeding. Fecal characteristics were scored by use of a dichotomous scale of 0 or 1 for grossly normal or diarrhea.

**Bacteriologic culture of fecal samples for detection of Salmonella organisms**—Sterile cotton-tipped applicators were used to collect fecal samples from each calf prior to challenge for qualitative detection of prechallenge Salmonella exposure. Fecal samples were collected from calves daily prior to challenge. The number of prechallenge fecal samples collected from calves varied according to age at the time of challenge. The minimum number of prechallenge bacteriologic cultures performed was 1, and the maximum was 3. Rectal swab specimens were collected for bacteria at time of diagnosis and cultured on Brilliant Green agar and on Brilliant Green agar plates were incubated for 24 hours at 37°C. Suspect colonies were subcultured to achieve a pure growth and tested by use of triple sugar iron, urea, and ONitrophenyl-b-d-galactopyranoside media biochemical tests. Quantitative Salmonella cultures of fecal samples were performed on study days 3, 6, 12, and 15. Prior to collection of fecal samples, 8 mL of sterile saline (0.9% NaCl) solution (0.13M) was placed in disposable sterile plastic tubes, which were then labeled and weighed. Approximately 3 g of feces was collected from the rectum of each calf by use of a disposable latex glove and placed in the respective labeled tube. Each tube was then reweighed and the contents homogenized and serially diluted in sterile saline solution to give two 10-fold dilutions and three 100-fold dilutions. One hundred microliters of the homogenized sample and each dilution were plated on Brilliant Green agar containing kanamycin (50 µg/mL) and Brilliant Green agar containing ceftiofur (8 µg/mL). Kanamycin was added to the media to improve the selectivity of the media for the challenge strain. Brilliant Green agar supplemented with ceftiofur (8 µg/mL) was inoculated similarly to detect Salmonella isolates with decreased susceptibility to ceftiofur. One milliliter of the original...
homogenized sample was also placed in 10 mL of tetrathionate enrichment media. After 24 hours of incubation at 37°C, the number of colonies present on each plate was counted, and the tetrathionate enrichments were plated on kanamycin-and ceftiofur-containing Brilliant Green agars. Three colonies from each call were tested with O antigen specific antiser on each day to determine the serogroup specificity of the Salmonella organism isolated. The number of Salmonella organisms present in the original fecal sample was calculated on the basis of the sample weight, dilution factor, and number of colonies counted. As a result of an oversight on day 18, sample tubes were not weighed following addition of feces. On this day, mean fecal sample weights were assigned to the 4 samples that had growth on the dilution plates to provide estimated quantitative bacteriologic culture results.

**Bacteriologic culture of tissue specimens for detection of Salmonella organisms**—Mesenteric lymph nodes and cecal contents were aseptically collected from each calf at necropsy. Approximately 2 g of each was placed into 8 mL of sterile saline solution (0.15M) in preweighed disposable sterile plastic tubes, which were then labeled and reweighed. A separate tube was used for each organ. The contents were homogenized and serially diluted in sterile saline solution to give two 10-fold dilutions and three 100-fold dilutions. One hundred microliters of the homogenized sample and each dilution were plated on Brilliant Green agar containing kanamycin (50 µg/mL) and Brilliant Green agar containing ceftiofur (8 µg/mL). One milliliter of the original homogenized sample was also placed in 10 mL of tetrathionate enrichment media. After 24 hours of incubation at 37°C, the number of colonies present on each plate was counted and the tetrathionate enrichment media plated on kanamycin-and ceftiofur-containing Brilliant Green agars. The MIC of ceftiofur was determined for all Salmonella organisms isolated at necropsy by use of the NCCLS methods. Nonserogroup B Salmonella isolates were sent to the Salmonella Reference Center for serotyping.

**Determination of ceftiofur MIC**—The MICs were determined by use of a commercially prepared dehydrated panel broth microdilution method that conforms to the guidelines recommended by the NCCLS as previously described. Cation supplemented Mueller-Hinton broth was used as the growth medium for all strains. In addition to the test strains, the following NCCLS-recommended American Type Culture Collection (ATCC) quality control strains were also tested: Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, and Pseudomonas aeruginosa ATCC 27893.

**Statistical analysis**—Mean age and total plasma protein concentrations of medicated and nonmedicated calves were compared by use of a student t test. Data from the 3 days prior to initiation of treatment were summed for each clinical parameter, and the relative rank of summed scores for medicated and nonmedicated calves were compared by use of the Mann-Whitney U test. Rectal temperature was classified as normal (≥ 37.7°C and ≤ 39.2°C) or abnormal (< 37.7°C or > 39.2°C). The published reference range for rectal temperature in calves is higher than the classification used for this analysis. The range of values considered normal in this analysis was the 95% confidence interval for age-matched healthy nonchallenged control calves (n = 18) from this and 2 other studies conducted within a 4-month period. We elected not to use published reference range values because the origin of these values is undefined, and 40% of measurements from healthy control calves were between 37.7 and 38.6°C, suggesting that the published reference range is not derived from calves of this age group or that the calves were raised under different environmental conditions. Fecal quantitative bacteriologic culture results failed to meet the conditions of normality with the Kolmogorov-Smirnov test. As such, the pretreatment fecal quantitative bacteriologic culture results of calves allocated to the medicated and nonmedicated groups were compared by use of the nonparametric Mann-Whitney U test.

Clinical data and fecal quantitative bacteriologic culture results following initiation of treatment reflected repeated measures of nonparametric data with uneven group sizes and censoring. Censoring reflected calf death or euthanasia. Both data sets were analyzed with the Wei-Lachin test. Analysis of fecal shedding of Salmonella organisms was based on the results of quantitative bacteriologic culture performed on study days 6, 9, 12, and 15. Day 18 results of fecal bacteriologic culture were excluded from the analysis, as they reflected an estimate as a result of the sample weighing error on this day. Clinical data following initiation of treatment were designated into 5 periods. Data from experimental days 5 to 7 were included in period 1, days 8 to 10 into period 2, days 11 to 13 in period 3, days 14 to 16 in period 4, and days 17 and 18 in period 5. Kaplan-Meier survival estimates were also calculated for each clinical parameter, and the survival curves of medicated and nonmedicated calves for each parameter following initiation of treatment were compared by use of the Peto log-rank test.

**Results**

**Pretreatment clinical comparisons**—Nine calves died or were euthanatized (ie, 7/36 challenged calves and 2/6 nonchallenged control calves) prior to initiation of treatment on day 4. Necropsies performed on the nonchallenged control calves determined that the 2 calves had died from enterotoxigenic E coli infection. During the 86 hours preceding the initiation of treatment, 22 of 29 (76%) of the surviving S Typhimurium-challenged calves had diarrhea, 4 (14%) had a fever, 11 (38%) had a decrease in appetite, and 9 (31%) had signs of a depressed mental attitude.

Mean age of medicated and nonmedicated calves was 2.4 days old (range, 1 to 4 days old) at the time of challenge, and the mean total serum protein concentrations for medicated and nonmedicated calves 24 days old was 6.6 g/dL (range, 5.7 to 7.5 g/dL). Total plasma protein concentrations varied significantly by treatment (P = .0001). The mean age and total plasma protein concentrations of medicated and nonmedicated calves were compared by use of a student t test. Data from the 3 days prior to initiation of treatment were summed for each clinical parameter, and the relative rank of summed scores for medicated and nonmedicated calves were compared by use of the Mann-Whitney U test. Rectal temperature was classified as normal (≥ 37.7°C and ≤ 39.2°C) or abnormal (< 37.7°C or > 39.2°C). The published reference range for rectal temperature in calves is higher than the classification used for this analysis. The range of values considered normal in this analysis was the 95% confidence interval for age-matched healthy nonchallenged control calves (n = 18) from this and 2 other studies conducted within a 4-month period. We elected not to use published reference range values because the origin of these values is undefined, and 40% of measurements from healthy control calves were between 37.7 and 38.6°C, suggesting that the published reference range is not derived from calves of this age group or that the calves were raised under different environmental conditions. Fecal quantitative bacteriologic culture results failed to meet the conditions of normality with the Kolmogorov-Smirnov test. As such, the pretreatment fecal quantitative bacteriologic culture results of calves allocated to the medicated and nonmedicated groups were compared by use of the nonparametric Mann-Whitney U test.

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hours following administration of the colostrum supplement were 5.8 and 5.7 mg/dL, respectively (range, 5.0 to 7.8 mg/dL). The mean body weight of medicated and nonmedicated calves prior to challenge was 47.6 ± 5 and 44.25 ± 8.1 kg, respectively. There were no significant differences in age, total plasma protein, and body weight between medicated and nonmedicated groups. Rectal temperature, appetite scores, and fecal characteristics were similar (P = 0.31 to 0.52) for medicated and nonmedicated calves prior to initiation of treatment. Despite random allocation of calves, those selected for medication had significantly (P = 0.067) worse attitude scores than nonmedicated calves during the 3 days prior to initiation of treatment. The pretreatment difference between groups reflected the allocation of the 2 calves with the highest attitude scores to the medicated group. Both of these calves subsequently died, the first 24 and the second 36 hours after initiation of treatment.

**Therapeutic drug monitoring**—The plasma concentration of ceftiofur and DFC metabolites in medicated calves remained higher than the ceftiofur MIC (1 µg/mL) for the S Typhimurium challenge strain up through 24 hours after the fifth treatment. Ceftiofur and DFC have similar or the same potency in vitro against Salmonella organisms. The lowest serum concentrations were observed 24 hours following the first dose of ceftiofur (day 4; mean, 2.78 µg/mL; range, 1.3 to 4.2 µg/mL; Fig 1). Plasma ceftiofur and DFC metabolite concentrations following dose administration on days 6 and 8 were slightly higher than on day 1 (mean, 4.0 µg/mL; range, 2.2 to 7 µg/mL).

**Posttreatment clinical comparisons**—Ceftiofur-medicated calves had significantly (period 1, P = 0.02; period 2, P = 0.03) fewer days of abnormal rectal temperatures, compared with nonmedicated control calves, for the 8 days following initiation of treatment (Fig 2). A significant (P = 0.031) difference was also observed in the hazard function for development of high or low rectal temperature following initiation of ceftiofur treatment (Fig 3). All of the nonmedicated calves had an abnormal rectal temperature between study days 5 and 18; in contrast, 4 of 15 medicated calves maintained a normal rectal temperature.

Calves medicated with ceftiofur had fewer days of diarrhea than nonmedicated control calves (Fig 2). A significant (P = 0.024) decrease in diarrhea was observed in periods 2 to 4 (days 8 to 16).

No significant differences were observed in the ranking of appetite and attitude scores between ceftiofur-medicated and nonmedicated calves (Fig 2). Ceftiofur-medicated calves lost less body weight than nonmedicated calves (0.72 and 3.04 kg, respectively); however, the difference in weight loss was not signifi-
cant \((P = 0.08)\). Nonchallenged control calves gained a mean value of 0.72 kg. Three of 15 medicated calves and 4 of 14 nonmedicated calves died or were euthanatized between days 4 and 18. The difference in mortality rates between groups (20 vs 28%) was not significant.

**Pretreatment fecal shedding of Salmonella organisms**—No Salmonella organisms were isolated from the qualitative bacteriologic culture of fecal samples performed on all calves prior to S Typhimurium challenge. The sensitivity of a single bacteriologic culture result is limited, and we presume that the subsequent posttreatment isolation of S enterica serovar Newport from 1 calf and S enterica serovar Anatum from a second calf reflect detection failure, as similar isolates were subsequently isolated from calves at the source dairy. On day 3 following oral S Typhimurium (TY1212) challenge, 90% (26/29) of the surviving calves were shedding the challenge strain in feces prior to the allocation of calves to medicated and nonmedicated control groups. On day 3 prior to allocation of calves to treatment and control groups, the mean number of Salmonella organisms shed in feces was \(1.45 \times 10^6\) CFU/g, with no significant difference in fecal shedding between groups.

**Posttreatment fecal shedding of Salmonella organisms**—A significant decrease in the number of Salmonella organisms and the proportion of calves shedding S Typhimurium was observed by quantitative bacteriologic culture on days 6, 9, and 12 (Fig 4 and 5). Shedding of Salmonella organisms in feces by nonmedicated calves increased until day 9, peaking at \(8.5 \times 10^6\) CFU/g. Shedding of Salmonella organisms in feces by ceftiofur-medicated calves declined by 3 log values to \(4.9 \times 10^3\) CFU/g on day 9. The mean peak number of Salmonella organisms shed in feces of calves that succumbed to infection \((2.9 \times 10^7 \text{ CFU/g})\) was significantly \((P = 0.01)\) greater than the mean peak number of Salmonella organisms shed in the feces of calves that survived challenge \((1.58 \times 10^6 \text{ CFU/g})\).

**Bacteriologic culture of tissue specimens for detection of Salmonella organisms**—Salmonella enterica serovar Typhimurium was isolated from specimens from 7 of 12 ceftiofur-medicated calves and from all 10 nonmedicated calves on day 18. Ceftiofur treatment was associated with a significant \((P = 0.01)\) decrease in Salmonella contamination of mesenteric lymph nodes and with a significant \((P = 0.049)\) decrease in the proportion of calves from which S Typhimurium was isolated from ≥ 1 site (Fig 5). No difference between medicated and nonmedicated calves was observed in the number of Salmonella organisms isolated from cecal contents and feces on day 18.

**Ceftiofur susceptibility**—The ceftiofur MIC for S Typhimurium (TY1212) recovered from ceftiofur-med-
icated and nonmedicated calves at necropsy was the same as the ceftiofur MIC for the inoculating challenge dose (0.5 to 1 µg/mL). *Salmonella* organisms with a ceftiofur MIC > 8 µg/mL were isolated from 2 calves during the study; S Newport was isolated from the feces of a medicated calf on day 6, and S Anatum was isolated from the feces of a medicated calf on day 18. Both isolates had a ceftiofur MIC of 16 µg/mL. These nonchallenge *Salmonella* isolates were only isolated from the 2 calves on 1 occasion. No other *Salmonella* serotypes were isolated from medicated or nonmedicated calves, and none of the *S* Typhimurium isolated had a decrease in ceftiofur susceptibility.

**Discussion**

There are no antimicrobials licensed for treatment of bovine salmonellosis in the United States. Antimicrobial treatment of salmonellosis in neonatal calves is therefore largely empirical as a result of the void of antimicrobial efficacy data available for this pathogen. Most of the antimicrobials licensed for use in livestock have been developed and evaluated for treatment of bovine respiratory disease. Consequently, the antimicrobial dose and dose administration frequency are formulated to maintain therapeutic drug concentrations against *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*. Antimicrobials licensed for parenteral administration in cattle include enrofloxacin (beef cattle only), penicillin, ampicillin, amoxicillin, ceftiofur, oxytetracycline, erythromycin, tylosin, tilmicosin, spectinomycin, sulfamethazine, and florfenicol. Of these, ampicillin, amoxicillin, ceftiofur, oxytetracycline, sulfamethazine, florfenicol, and enrofloxacin have a gram-negative spectrum of activity suitable for treatment of *Salmonella* infections. A number of constraints further limit the choice of antimicrobial drugs for treatment of salmonellosis in neonatal calves. Extralabel use of enrofloxacin is illegal, protracted drug residues limit the application of aminoglycosides, and antimicrobial resistance often limits the application of oxytetracycline, sulfamethoxine, amoxicillin, and ampicillin. Cephalosporin resistance among *Salmonella* organisms has been more recently documented, particularly in S Newport isolates from dairy cattle.

Prudent or judicious antimicrobial use guidelines have been proposed to preserve efficacy of antimicrobial compounds for maintenance of human and animal health. Therapeutic efficacy and antimicrobial pharmacokinetic data are required to practice science-based prudent drug use. The objective of our study was to investigate the efficacy of ceftiofur used at a high extralabel dose for treatment of salmonellosis in neonatal calves. In our experiment, antimicrobial treatment was initiated on day 4 (ie, 84 hours following *Salmonella* challenge). The onset of antimicrobial treatment was planned to mimic field application where therapeutic drug use is initiated following the onset of clinical disease.

The label dose of ceftiofur for treatment of respiratory disease in cattle is 1.1 to 2.2 mg/kg. For ceftiofur, the minimal concentration that inhibits 90% (MIC₉₀) of *M* haemolytica is 0.015 to 0.06 µg/mL. The MIC₉₀ of ceftiofur for *Salmonella* spp is 1 µg/mL, approximately 40 times that of the label dose target pathogen. The extralabel high dose (5 mg/kg) of ceftiofur selected in our experiment was based on the MIC₉₀ of ceftiofur for *Salmonella* spp and the pharmacokinetics of ceftiofur in the bovine neonate. Consistent with other water-soluble antimicrobial drugs, ceftiofur has a higher volume of distribution in neonatal calves, compared with adult cattle. Peak plasma concentrations are subsequently lower in neonates than adults following equivalent dose administration. Hence, a slightly larger dose is required to achieve similar drug concentration in neonates. As with other β-lactam antimicrobials, the most significant factor determining efficacy is the time drug levels exceed the MIC of the target pathogen; therefore, the therapeutic objective is to maintain drug concentration above the MIC for the target pathogen for the duration of treatment.

The MIC of ceftiofur for the *S* Typhimurium challenge strain used in our experiment was 1 µg/mL. In vivo, ceftiofur is rapidly metabolized to DFC, a microbiologically active metabolite retaining the β-lactam ring. Desfuroylceftiofur has an exposed sulfhydryl moiety that is involved in reversible binding to cysteine moieties, glutathione, sulfhydryl, and itself (DFC-dimer) through formation of disulfide bonds. The exposed sulfhydryl moiety of DFC is also responsible for reversible covalent binding of DFC to plasma and tissue proteins, with the formation of disulfide or thioester bonds. The protein binding extends the biological half-life of DFC, since it protects the β-lactam ring from cleavage and reduces the rate of excretion by the liver and kidney. These conjugates all have intact β-lactam rings and can be reduced back to DFC. According to previous findings for *Salmonella* organisms and other organisms of veterinary importance, the difference between the MIC of DFC and ceftiofur is not detectable; hence, we would expect an MIC of DFC of 1 µg/mL for the *S* Typhimurium challenge strain in our experiment. The combined concentration of ceftiofur and DFC may be expressed as ceftiofur equivalents. In healthy calves, a dose of 5 mg/kg provides a plasma ceftiofur equivalent concentration of > 1 µg/mL for 24 hrs. The assay used in our experiment measures the concentration of ceftiofur and its active metabolites.

Results of drug monitoring performed in our experiment revealed similar pharmacokinetics in calves with salmonellosis and maintenance of a therapeutic concentration of ceftiofur for the duration of the 5-day treatment period. Slightly higher plasma drug concentrations on days 6 and 8 likely reflect incomplete clearance of the drug within the 24-hour dose administration interval and might be expected in young animals.

The challenge dose of *S* Typhimurium selected was chosen for its capacity to induce clinical disease and fecal shedding of *Salmonella* organisms without producing high mortality rate. Nine calves died or were euthanatized prior to initiation of treatment. The death of 2 nonchallenged control calves infected with enterotoxigenic *E coli* suggests that some of the pretreatment deaths in challenged calves may have been caused by this pathogen. It is unlikely that enterotoxigenic *E coli*...
impacted the results of the therapeutic trial, as the mean age of the calves at the onset of antimicrobial treatment was 6.4 days.

The use of antimicrobials for treatment of salmonellosis has historically been controversial as a result of concerns regarding efficacy, induction of persistent fecal shedding, and selection for antimicrobial resistance. In human medicine, antimicrobial treatment is generally reserved for treatment of invasive salmonellosis. Bacteremia is a common feature of salmonellosis in neonatal calves, and disease outbreaks are commonly associated with high morbidity and mortality rates. In our therapeutic trial, a rapid positive therapeutic effect in the form of attenuated clinical signs and a decrease in fecal shedding of Salmonella organisms were observed in ceftiofur-medicated calves. The significant attenuation of fever and diarrhea was accompanied by a decrease in weight loss and a lower mortality rate, although the decreases in weight loss and mortality rate were not significant. Ancillary supportive care in the form of fluid treatment and nonsteroidal anti-inflammatory drugs was not included in our trial to avoid introduction of potentially confounding variables. Further improvement in clinical outcomes would be expected with provision of supportive treatments.

Following cessation of treatment, 3 medicated calves resumed fecal shedding of Salmonella organisms. Resumption of and prolonged fecal shedding of Salmonella organisms have been documented following the discontinuation of antimicrobial treatment in human and in poultry trials. The results of these studies contrast with those of most studies in humans, pigs, and cattle that have reported either no difference or a decrease in fecal shedding following antimicrobial treatment or a decrease in fecal shedding. Salmonella infections in poultry are generally not associated with clinical disease. These studies subsequently do not reflect a therapeutic application of antimicrobials in the treatment of salmonellosis in livestock. The prolific fecal shedding of Salmonella organisms by severely compromised calves observed in our study illustrates the relationship between host compromise and fecal shedding of Salmonella organisms. The resumption of fecal shedding by 3 medicated calves and the proportion of medicated calves shedding Salmonella organisms in feces at the conclusion of our study appear to parallel reports in poultry. However, at the conclusion of the 18-day study, the number of organisms that were shed by medicated and nonmedicated calves was declining, and the composite results of Salmonella cultures of fecal samples and necropsy specimens indicated that all nonmedicated calves were still infected with Salmonella organisms, whereas S Typhimurium was isolated from 7 of 12 medicated calves.

The MIC of ceftiofur remained constant for the S Typhimurium challenge strain. However, 2 unrelated Salmonella serotypes with ceftiofur MICs of 16 µg/mL were isolated from calves during the course of the experiment. We suspect the source of these isolates was the dairy of origin because similar isolates were subsequently isolated from calves at the dairy. The presence of these isolates highlights the need to evaluate antimicrobial MICs prior to initiating herd-level therapeutic interventions. Failure to maintain antimicrobial drug concentrations above the MIC for an infecting organism is sometimes a limitation in the application of older β-lactam antimicrobials. In our experiment, the dose of ceftiofur administered maintained plasma ceftiofur concentrations above the MIC for the challenge strain. Salmonella organisms are facultative intracellular pathogens. Beta-lactam antimicrobials do not distribute widely into cells; therefore, plasma ceftiofur concentrations would be higher than intracellular concentrations.

Implementation of good management practices to prevent neonatal salmonellosis and other calf diseases is prudent from a business and animal welfare perspective. High mortality rates and compromised animal welfare are observed when prevention strategies fail. The results of our experiment indicate that treatment of salmonellosis in neonatal calves with a high extralabel dose (5 mg/kg) of ceftiofur promotes animal welfare, may reduce fecal shedding of Salmonella organisms, and may promote clearance of Salmonella infections in calves when the plasma ceftiofur concentration is maintained above the MIC for infective strain.

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