Efficacy of ceftiofur sodium for the control of mortality in neonatal pigs orally inoculated with K88+ (F4+) enterotoxigenic *Escherichia coli*

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Summary

Objective: To evaluate the efficacy of ceftiofur sodium (Naxel/Excenel Sterile Powder; Pharmacia & Upjohn, Kalamazoo, Michigan) administered IM once daily at 3 or 5 mg ceftiofur per kg BW for 3 days for control of mortality associated with K88+ (F4+) *Escherichia coli*-induced colibacillosis in neonatal piglets.

Methods: Pregnant gilts susceptible to colibacillosis associated with K88+ *E coli* were farrowed in farrowing crates with solid walls to prevent contact between litters. A total of 29 gilts with 240 piglets were enrolled, with litters randomly assigned to study group by order of farrowing. Groups included uninoculated-no treatment, inoculated-sterile saline IM, inoculated-ceftiofur sodium IM (3 mg per kg), and inoculated-ceftiofur sodium IM (5 mg per kg). Treatments were administered on Days 0, 1, and 2. On Day 0, each inoculated piglet received 20 mL inoculum orally, containing approximately 10^8 colony forming units per mL of *E coli* strain M1823B (O157:K88ac:H43). Clinical observations were conducted twice daily for 8 days.

Results: Mean mortality rates for the two ceftiofur-treated groups were lower than for the inoculated-saline group (P=.003). The proportion of normal stool scores was higher in ceftiofur-treated groups than in the inoculated-saline group ($P \leq .008$). Average daily gain for surviving piglets did not differ among the groups.

Implications: Ceftiofur sodium administered IM daily for 3 consecutive days at 3 or 5 mg per kg significantly reduced mortality and increased percentage normal stool scores in treated piglets inoculated with K88+ *E coli*, compared to untreated, inoculated piglets.

Keywords: swine, bacteria, *Escherichia coli*, enterotoxins, diarrhea

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Diarrhea occurring in piglets within the first few days after birth (0 to 4 days) is often caused by *Escherichia coli* infection of the jejunum and ileum.[1] The disease is economically important in the swine industry due to the losses observed in the intensive management systems currently used in farrowing houses.[1] Causative bacteria adhere to the epithelium of the small intestine by means of fimbrial adhesins and produce one or more enterotoxins that may increase fluid secretion in the small intestine up to tenfold, causing diarrhea if excess fluid is not absorbed in the large intestine.[1] No primary inflammatory reactions occur in this type of hypersecretory neonatal diarrhea.[1] However, the ability of the large intestine to compensate for sudden fluid flux is easily overwhelmed in neonates.[1]

Antibiotic therapy is indicated for the treatment of neonatal diarrhea caused by enterotoxigenic *E coli,* such as K88+ (F4+) strains.[1] Therapy should be rapidly instituted before piglets become severely dehydrated and should seek to inhibit growth of enterotoxigenic bacteria in the small intestine. Fluid and electrolyte replacement is useful in treating dehydration and acidosis. Antibacterial treatment should be coupled with appropriate husbandry techniques, which include providing a draft-free, warm environment, using farrowing crates designed to keep the piglets away from fecal material, and maintaining appropriate sanitation of the sow and the farrowing facility.[1]

Ceftiofur sodium (Naxcel/Excenel Sterile Powder; Pharmacia & Upjohn, Kalamazoo, Michigan) is currently approved in the United States and many other countries for the control and treatment of swine respiratory disease (SRD), administered IM at 3 or 5 mg ceftiofur per kg BW for 3 consecutive days. However, its spectrum of activity and pharmacokinetic and pharmacodynamic characteristics, including tissue homogenate data, support a clinical rationale for use of ceftiofur sodium in the treatment of enterotoxigenic colibacillosis in neonatal piglets. Ceftiofur was confirmed to be an effective treatment for colibacillosis in an enterotoxigenic K99+ *E coli* challenge model study[2] and in field studies where oral[3] and IM[4] administration were evalu-

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ated for the treatment of naturally occurring colibacillosis. The present study evaluated the efficacy of ceftiofur sodium administered IM for 3 consecutive days at 3 or 5 mg ceftiofur per kg BW for the control of mortality associated with secretory diarrhea in neonatal piglets orally inoculated with K88+ *E coli*.

Materials and methods

Experimental design

Gilts were obtained and placed in standard farrowing crates at least 14 days before their expected farrowing date. Litters (n=29) were randomly assigned in order of farrowing to one of four study groups (Table 1). Birth weights were obtained when piglets were processed at the end of farrowing. Piglets in Groups 2, 3, and 4 were inoculated within 7 hours of the end of farrowing (Day 0). Clinical signs were observed twice daily, beginning 12 hours after the end of farrowing, and through one observation on Day 8. The first treatment was administered to all designated piglets 6 hours after inoculation on Day 0, regardless of clinical signs. The first clinical evaluation was made either before or after the first treatment, depending on the time of inoculation. Day 1 and Day 2 treatments were administered at 24-hour intervals. The personnel who conducted the clinical evaluations and necropsies remained blinded to the assigned treatments throughout the trial. The trial was conducted according to the *Guidelines for Good Target Animal Study Practices*[5] and was consistent with applicable laws and regulations governing the humane care of animals.

Study animals

Pregnant Lancaster or Large White cross gilts (n=31) that had not been vaccinated against *E coli* were obtained from a source that produced piglets susceptible to enterotoxigenic diarrhea associated with K88+ *E coli*, with litter mortality rates between 0 and 60% (unpublished data). Two groups of gilts (n=11 and n=20) were placed in standard farrowing crates (with solid walls to prevent nose-to-nose contact between litters) to acclimatize for at least 14 days before their expected farrowing date. Gilts were observed every 2 to 4 hours to assist in farrowing as needed and to document farrowing times. Cross-fostering was allowed before inoculation to balance litter size. All piglets with obvious physical problems, injuries, or birth weight <0.9 kg were excluded from the study, as was any piglet born after the litter had been inoculated.

Table 1: Study conditions for groups of neonatal pigs orally inoculated with *Escherichia coli*[1] within 7 hours of farrowing and treated IM with ceftiofur sodium[2] for 3 consecutive days beginning on the day of inoculation

	Group 1	Group 2	Group 3	Group 4
No. of litters (pigs)	6 (49)	7 (60)	8 (67)	8 (64)
Inoculated with *E coli*	No	Yes	Yes	Yes
Treatment	None	Saline	Ceftiofur sodium	Ceftiofur sodium
Dosage of ceftiofur (mg/kg BW)	NA[3]	NA	3	5

1 Inoculum for each piglet contained 2-4 × 10^9 colony forming units of *E coli* strain M1823B (O157:K88ac:H43).
2 Naxcel Sterile Powder; Pharmacia & Upjohn, Kalamazoo, Michigan.
3 NA = not applicable

During the entire acclimatization and study periods, the only antibacterials administered to the piglets were the experimental ceftiofur treatments included in the study design. No antibacterials were administered to the gilts during the acclimatization and study periods.

Administration of treatments

Ceftiofur sodium was diluted to 10 mg ceftiofur per mL for accurate dosing and administered IM using a 20-gauge, 2.5-cm sterile needle. The first treatment (Day 0) was administered in the left neck, and Day 1 and Day 2 treatments were administered in the right and left neck, respectively.

Inoculum

Each inoculated piglet received 20 mL of inoculum orally, containing approximately 10^8 colony forming units (CFUs) per mL of *E coli* strain M1823B (O157:K88ac:H43) suspended in trypticase soy broth. This isolate, a hemolytic strain originally isolated from the intestine of a pig with post-weaning diarrhea, produces the heat labile enterotoxin (LT) and the heat stable toxin (STb).[6] The ceftiofur minimum inhibitory concentration (MIC) for this organism is 0.5 μg per mL. The inoculum was administered in 10-mL portions, with all piglets in a litter receiving the first 10 mL before any piglet received the second 10 mL. Inocula were administered orally with the aid of a 13-cm, 14-gauge catheter (Angiocath; BD Medical Systems, Franklin Lakes, New Jersey) with the stylet removed. A new catheter was used for each litter. Throughout the study, personnel changed gloves after handling a litter and before moving to the next, to minimize the possibility of spreading the challenge organism between litters. In addition, care was taken when cleaning crates to avoid splashing fecal material into adjacent crates.

Clinical observations and scoring

Observers recorded weights, determined a stool score using the scoring system described in Table 2, and noted an illness index score and other relevant clinical observations. Clinical scoring was performed by evaluators who remained blinded to the assigned study groups throughout the study.

Table 2: Stool scoring system for neonatal pigs orally inoculated with *Escherichia coli*[1] within 7 hours of farrowing and treated IM with ceftiofur sodium[2] for 3 consecutive days beginning on the day of inoculation

Score	Stool consistency
0	Normal, solid feces
1	Semi-solid feces
2	Watery feces with some solid material
3	Profuse watery feces with little or no solid material

1 Inoculum for each piglet contained 2-4 × 10^9 colony forming units *E coli* strain M1823B (O157:K88ac:H43).
2 Naxcel Sterile Powder; Pharmacia & Upjohn, Kalamazoo, Michigan.

Moribund or savaged piglets were euthanized. All piglets removed from the study or found dead were necropsied. Deaths were categorized as due to colibacillosis if evidence of dehydration was present at necropsy.

Mortality due to enterotoxigenic colibacillosis was calculated as the proportion of piglets in a litter that died due to dehydration associated with enterotoxigenic colibacillosis. Percent normal stool scores was calculated for each surviving piglet as the number of normal stool scores divided by the number of stool evaluations for that piglet. Average daily gain was calculated for the surviving piglets as the final weight of each piglet minus the birth weight, divided by the number of days the piglet was on study.

No rectal swabs, fecal samples, or tissue samples were collected for culturing during this study.

Statistical analysis

Data from all piglets that died or were euthanized prior to inoculation, or that were born after the rest of the litter was inoculated, were excluded from the analysis data set. The primary variable in this randomized block design study was mortality associated with dehydration due to enterotoxigenic colibacillosis. Stool scores and ADG were secondary variables. Mortality was analyzed as a general linear mixed model using the PROC MIXED procedure from SAS.[7] Mortality was expressed as the Freeman-Tukey angularly transformed proportion of piglets within a litter that died due to enterotoxigenic colibacillosis (using n + 0.5 as weights, where n = number of piglets assigned to the study within the litter). The mixed model included the fixed effects of period (farrowing period for the two groups of gilts), treatment, and period by treatment interaction, and the random effect of gilt-litter within period by treatment. The random effect of gilt-litter within period was used as the error term to test the fixed effects of treatment.

Stool scores for the surviving piglets were analyzed as a general linear mixed model using the PROC MIXED procedure from SAS. Stool scores were expressed as the Freeman-Tukey angularly transformed proportion of normal stool scores for each piglet (using n + 0.5 as weights, where n = number of stool evaluations). The mixed model included the fixed effects of period (farrowing period), treatment, and period by treatment interaction, and the random effects of gilt-litter within period by treatment and residual. The random effect of gilt-litter within period was used as the error term to test the fixed effects of treatment.

The ADG for surviving piglets was analyzed as a general linear mixed model using the PROC MIXED procedure from SAS. The mixed model included the fixed effects of period (farrowing period), treatment, and period by treatment interaction, and the random effects of gilt-litter within period by treatment and residual. The random effect of gilt-litter within period was used as the error term to test the fixed effects of treatment.

For each variable, pairwise comparisons were made between Groups 3 and 4 and Group 2. The farrowing period by treatment interaction for each comparison was accomplished through contrast statements. No statistical comparisons were made between Group 1 and the other study groups or between Groups 3 and 4. All analyses were conducted using α=.05.

Table 3: Mortality, proportion of normal stool scores, and ADG of surviving piglets in groups of neonatal pigs orally inoculated with *Escherichia coli*[1] within 7 hours of farrowing and treated IM with ceftiofur sodium[2] for 3 consecutive days beginning on the day of inoculation

	Study Group[3]			
	1 (n)	**2 (n)**	**3 (n)**	**4 (n)**
Mean mortality rate[4] (%)	2.0 (49)	48.3 (60)	6.0[a] (67)	6.2[a] (64)
Normal stool scores[5] (%)	87.2 (48)	72.2 (60)	90.6[b] (65)	89.1[c] (62)
ADG[6] (kg/day)	0.181 (48)	0.186 (60)	0.197 (65)	0.194 (62)

1 Inoculum for each piglet contained 2-4 × 10^9 colony forming units *E coli* strain M1823B (O157:K88ac:H43). Piglets were inoculated on Day 0, and observed for clinical signs, stool scores, and mortality once on Days 0 and 8, and twice on Days 1-7.

2 Naxcel Sterile Powder; Pharmacia & Upjohn, Kalamazoo, Michigan.

3 Group 1: no challenge, no treatment; Group 2: challenge, sterile saline for 3 days; Group 3: challenge, ceftiofur sodium, 3 mg/kg for 3 days; Group 4: challenge, ceftiofur sodium, 5 mg/kg for 3 days.

4 Calculated as the proportion of piglets in a litter that died due to dehydration associated with enterotoxigenic colibacillosis, and analyzed as a general linear mixed model using the PROC MIXED procedure from SAS. Mortality was expressed as the Freeman-Tukey angularly transformed proportion of piglets within a litter that died due to enterotoxigenic colibacillosis (using n + 0.5 as weights, where n = number of piglets assigned to the study within the litter).

5 Determined as the number of normal stool scores divided by the number of stool evaluations, and analyzed as a general linear mixed model using the PROC MIXED procedure from SAS. Stool scores were expressed as the Freeman-Tukey angularly transformed proportion of normal stool scores for each piglet (using n + 0.5 as weights, where n = number of stool evaluations).

6 Final weight for each piglet minus the birth weight, divided by the number of days on study, analyzed as a general linear mixed model using the PROC MIXED procedure from SAS.

a Different from Group 2 (*P*=.003)

b Different from Group 2 (*P*=.006)

c Different from Group 2 (*P*=.008)

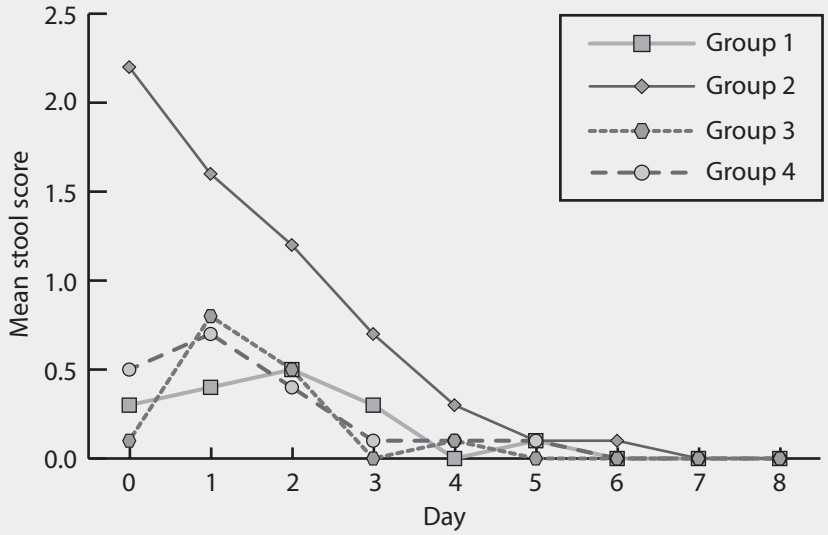
Results

A total of 29 gilts with 240 piglets were included in the statistical analyses. Data from five piglets whose deaths were not related to colibacillosis were excluded before the analyses of stool scores and ADG.

Results of analysis of mean mortality due to colibacillosis, proportion of normal stool scores in surviving piglets, and ADG in surviving piglets are presented in Table 3. Mean mortality rates for Groups 3 and 4 were lower than for Group 2, and the proportion of normal stool scores during the entire study period was higher in

Groups 3 and 4 than in Group 2. Daily stool scores (which were not analyzed statistically) were numerically higher through Day 4 for Group 2 compared to Groups 3 and 4 (Figure 1). There were no significant differences in ADG between Group 2 and Groups 3 or 4 ($P>.05$).

Figure 1: Mean daily stool scores for four groups of neonatal pigs (Table 1) orally inoculated with *Escherichia coli*, strain M1823B (O157:K88ac:H43), 2-4 × 10^9 colony forming units within 7 hours of farrowing (Day 0) and treated IM with ceftiofur sodium for 3 consecutive days beginning on Day 0. Clinical signs were observed twice daily, beginning 12 hours after the end of farrowing through one observation on Day 8. The stool scoring system is described in Table 2.



Discussion

The results of this study provide clinical confirmation of the effectiveness of ceftiofur for the treatment of enterotoxigenic colibacillosis in neonatal piglets. These data are consistent with the pharmacokinetics and pharmacodynamics of ceftiofur.[8]

Salmon et al[9] determined ceftiofur MIC for 84 swine *E coli* isolates using the microdilution method of the National Committee on Clinical Laboratory Standards with appropriate quality control strains. The MIC_{50} (which represents the MIC for the most sensitive 50% of the isolates) was 0.5 µg per mL and the MIC_{90} (which represents the MIC for the most sensitive 90% of the isolates) was 1.0 µg per mL (range 0.25 to 4.0 µg per mL, mode 0.5 µg per mL). These values are comparable to the ceftiofur MICs for *Salmonella* serovar Choleraesuis, the least ceftiofur-sensitive pathogen associated with SRD for which ceftiofur sodium and ceftiofur hydrochloride have label claims.[10,11] In addition, 885 swine enteric isolates, including *E coli*, collected in Missouri in 1996-1997, were susceptible to ceftiofur.[12] The ceftiofur MIC for the enterotoxigenic *E coli* inoculation strain used in the present study was 0.5 µg per mL, in agreement with the mode and MIC_{50} of previously reported swine *E coli* isolates.[9]

In a pharmacokinetic study evaluating the plasma concentrations of ceftiofur sodium or ceftiofur hydrochloride in pigs,[8] the critical pharmacokinetic parameters (maximum plasma concentration achieved after treatment administration, area under the plasma concentration-time curve, and time the plasma concentration remained above 0.2 µg per mL) were similar for pigs receiving either ceftiofur sodium or ceftiofur hydrochloride in the same dosage. The dosing regimens tested for the treatment of colibacillosis in our study included the range of dosing regimens approved by the Food and Drug Administration Center for Veterinary Medicine for the treatment and control of swine bacterial respiratory disease.

A tissue distribution study conducted in pigs provides further evidence supporting the effectiveness of ceftiofur for enteric colibacillosis.[13] Pigs received a single IM dose of ceftiofur sodium at 3 mg ceftiofur per kg BW, the lower end of the dose range approved in the United States for the treatment of SRD. Ceftiofur concentrations in the washed homogenate of the distal 1 m of jejunum and in mesenteric lymph node were 1.00 and 1.09 µg per g, respectively, 7.5 hours after treatment.

Although a formal dose proportionality study evaluating the pharmacokinetics of ceftiofur when administered at several incremental dose levels has not been conducted in swine, previous studies support dose proportionality in swine.[14] Assuming dose proportionality, the tissue concentrations in the distal portion of the jejunum would be expected to remain above the MIC_{90} for the *E coli* strains tested[9] for approximately 18.5 to 19.5 hours after a single IM dose of 5 mg ceftiofur per kg BW, and tissue concentrations are estimated to be approximately 0.68 µg ceftiofur per mL at the 24-hour dosing interval. Since current technology cannot accurately measure the amount of ceftiofur that bacteria encounter at the site of colonization, namely, the luminal surface of the small intestine, it is not technically feasible to determine the ratio of the drug concentration at the site of infection to the MIC. While therapeutic levels of ceftiofur-related residues were detected in tissue samples of the small intestine[13] when pigs were administered ceftiofur, rapid degradation of ceftiofur in the large intestine and fecal material of animals[15,16] would likely limit its effectiveness against infections in the large intestine.

The results of this inoculation study confirm and extend previous work that evaluated the efficacy of ceftiofur for the treatment of colibacillosis. In a similar study conducted with a $K99^+$ *E coli*, a single dose of ceftiofur hydrochloride at 0.5 or 2.0 mg ceftiofur per kg BW was administered IM to piglets 6 hours after inoculation.[2] In pigs treated with either dose compared to untreated controls, mortality and abnormal stool scores were significantly lower, there was significantly less shedding of the challenge organism, and weight gain was significantly greater. Groups treated orally were also included in this study and a second challenge study.[2] All oral treatment regimens reduced mortality and abnormal stool scores and improved weight gain relative to untreated controls. The efficacy of the oral treatments

was later confirmed in two field studies.[3,4] In a multilocation field efficacy study[3] of ceftiofur for treatment of naturally occurring colibacillosis, a single oral dose of 13.5 mg ceftiofur per pig was effective, ie, reduced mortality and diarrhea in the surviving piglets compared with placebo-treated controls. The oral use of ceftiofur is no longer considered to be prudent use of a third generation cephalosporin. A second field study evaluated IM single-dose and 3-day regimens of 8 mg ceftiofur per kg (pigs <10 days old) and 11 mg ceftiofur per kg (pigs ≥10 days old) compared to untreated controls.[4] Clinical recovery rates and weight gains were higher for ceftiofur-treated groups. The efficacy demonstrated in these clinical studies supports the conclusion that ceftiofur is present at the mucosal surface of the small intestine at concentrations adequate for the treatment of colibacillosis in pigs.

While the present study provided clinical confirmation of the efficacy of ceftiofur for reduction of mortality associated with enterotoxigenic colibacillosis in neonatal swine, it is important to note that this is extra-label use of ceftiofur. A dose titration has not been performed for this use of ceftiofur, and the doses tested in the present study were those currently approved by the FDA and other regulatory bodies worldwide for the treatment and control of bacterial SRD. Although there are no systematic sampling studies currently conducted to evaluate trends in enterotoxigenic *E coli* antimicrobial resistance, data suggest that resistance may develop in some swine pathogens.[17] This emphasizes the importance of having a definitive diagnosis and knowledge of the MIC(s) for the pathogens prior to initiation of antimicrobial treatment. These clinical data are provided for practitioner information only to assist in the decision-making process regarding treatment selection.

Implications

- Under the conditions of this study, in neonatal pigs orally inoculated with K88+ *E coli,* mortality was lower and the percentage of normal stool scores was higher in groups treated IM with ceftiofur sodium (3 or 5 mg ceftiofur per kg BW) compared to groups that received no ceftiofur treatment.
- Ceftiofur sodium (Naxcel/Excenel Sterile Powder; Pharmacia & Upjohn) administered IM at the dose labeled for the treatment of SRD appears to be an effective treatment for reducing losses due to neonatal pig diarrhea caused by K88+ enterotoxigenic strains of *E coli.*

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