ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1981, p. 562-566
0066-4804/81/040562-05$02.00/0

Vol. 19, No. 4

Cephamycin C Treatment of Induced Swine Salmonellosis

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Received 4 September 1980/Accepted 29 January 1981

Weanling pigs in groups of 12 were infected orally with *Salmonella cholerae-suis* and were treated intramuscularly with doses of cephamycin C ranging from 12.5 to 337.5 mg twice daily for 10 days beginning 1 day postinoculation. Pigs in two other infected groups either received 300 mg of tetracycline orally on a similar schedule or served as nonmedicated controls. Optimal responses to cephamycin C were achieved at a twice daily dose of 112.5 mg. With this regimen, the febrile response was significantly reduced on day 2 and eliminated by day 5 postinfection, and the shedding of *Salmonella* spp. in feces was eliminated by day 7 postinfection; essentially, no lesions were found in the gastrointestinal tract at necropsy (day 26 postinfection). There was no mortality among recipients of the 112.5-mg dose; diarrhea was present on only 2% of the observation days. In contrast, 83% of the infected, nonmedicated pigs and 25% of the tetracycline-medicated pigs died, and diarrhea was present in these groups on 63 and 54% of the observation days, respectively. The striking benefits of cephamycin C treatment were achieved without adverse reactions. The weight gain and feed efficiency of the infected pigs treated with the 112.5-mg dose of cephamycin C and the noninfected, nonmedicated control pigs were equivalent.

Cephamycin C, a β-lactam antibiotic produced by *Streptomyces lactamdurans*, has a broad spectrum of activity against gram-negative organisms in vitro (7) and in vivo (8) and is resistant to degradation by β-lactamases (3). It has recently been shown to be highly effective in treating induced enterotoxigenic colibacillosis (scours) in both calves and piglets (6). This report is concerned with the activity of cephamycin C against an experimentally induced *Salmonella cholerae-suis* infection in weanling swine.

MATERIALS AND METHODS

Facilities, animals, and treatments. This experiment was conducted in the facilities of AMBICO, Inc., Dallas Center, Iowa. Eighty-four cross-bred pigs (North Central Iowa Pork Producers, Inc., Clear Lake, Iowa), all free of *Salmonella* spp., were used. The pigs, 5 to 6 weeks of age with a weight range of 6.4 to 12.6 kg (mean weight, 9.5 kg), were distributed by litter, sex, and weight into 14 isolation pens of six pigs each for infection and treatment according to the schedule shown in Table 1.

Cephamycin C-monosodium salt (Merck & Co., Inc., Rahway, N.J.) and oxytetracycline (Terramycin Soluble Powder Concentrate; Pfizer, Inc., New York, N.Y.) were used. Solutions were prepared at Merck & Co., Inc., color coded, and shipped to AMBICO, Inc. All drug treatments were administered twice daily (8:00 a.m. and 4:00 p.m.) for a total of 10 days beginning one day postinoculation (PI). The experiment was terminated on day 26 PI.

Diets. Pigs were fed a corn, soybean meal, grower-type ration free of antibacterial agents and *Salmonella* spp. Pigs had free access to the ration and water at all times.

***Salmonella* strain and challenge.** Strain 77-1547 of *Salmonella cholerae-suis* var. *kunzendorf* (herein referred to as *S. cholerae-suis*), obtained from Billie O. Blackburn of the National Animal Disease Laboratory in Ames, Iowa, was used in this experiment. This strain had been identified as the pathogen in an epizootic that resulted in the deaths of 52 pigs in a herd of 175. In the standardized disk susceptibility test (Kirby-Bauer method) (1), this strain was found to be susceptible to tetracycline (Te-30) but resistant to penicillin (P-10) and triple sulfa (SSS-.25). The minimal inhibitory concentration of cephamycin C was 4 μg/ml. The pigs were inoculated individually by feeding each a milk-ground corn mixture containing 2.0×10^9 colony-forming units of *S. cholerae-suis*.

Clinical parameters. The following health records were maintained over the course of the experiment: (i) individual weights, recorded on the day allotted to groups (2 days before inoculation), on the day the pigs were inoculated, and on day 26 PI; (ii) rectal temperatures, recorded daily for each pig beginning 1 day before inoculation and continuing to day 14 PI; (iii) fecal consistency, recorded daily as diarrhetic or solid for each pig throughout the experiment; (iv) feed consumption, recorded on day 26 PI; and (v) necropsy results; all pigs were necropsied when they died or after they were killed on day 26 PI.

TABLE 1. *Schedule for infection and treatment of pigs*

Pen no.	No. of pigs	*S. cholerae-suis*	Medication (mg/pig b.i.d.)
7, 13	6, 6	Inoculated	None
8, 14	6, 6	Inoculated	Tetracycline, 300 (oral)
4, 11	6, 6	Inoculated	Cephamycin C, 12.5 (IM)[a]
6, 10	6, 6	Inoculated	Cephamycin C, 37.5 (IM)
5, 12	6, 6	Inoculated	Cephamycin C, 112.5 (IM)
3, 9	6, 6	Inoculated	Cephamycin C, 337.5 (IM)
1, 2	6, 6	Noninoculated	None

[a] IM, Intramuscularly administered.

***Salmonella* spp. isolations.** Two rectal swabs from each pig were examined for *Salmonella* spp. before inoculation with *S. cholerae-suis*. Rectal swabs from each pig were examined for *Salmonella* spp. on days 7, 14, and 21 PI. At necropsy four tissue samples from each pig, including ileocecal lymph node, liver, spleen, and colon swab, were examined for *Salmonella* spp. Feed, fecal, or tissue samples were streaked directly onto xylose lysine, brilliant green agar and incubated for 24 h. at 37°C. Colonies suspected of being *Salmonella* spp. were picked and inoculated into triple sugar iron agar and lysine iron agar tubes and incubated at 37°C for 24 h. Serological typing was then used to determine the *Salmonella* serogroup.

Numbers of inoculated *S. cholerae-suis* in feces at day 2 postinoculation. Freshly collected fecal samples (1 g each) were homogenized in 9 ml of 0.01 M phosphate-buffered saline (pH 7.2) and diluted serially in this fluid; 0.1-ml quantities of 10^{-2}, 10^{-4}, and 10^{-6} dilutions were plated in duplicate on xylose lysine, brilliant green agar. The numbers of colonies of *S. cholerae-suis* on these plates were determined after a 24-h incubation at 37°C. Where available, five clones from each fecal sample suspected of being *Salmonella* spp. were picked from the xylose lysine, brilliant green agar plates and inoculated into triple sugar iron agar and lysine iron agar tubes and incubated at 37°C for 24 h. Serological typing was then used to assure the isolates were *Salmonella* serogroup C.

Lesions at necropsy. All pigs, when they died or were killed at the end of the experiment, were examined for macroscopic lesions typical of salmonellosis. Pigs dead at 8:00 a.m. were sufficently well preserved to allow examination for macroscopic lesions. The lesions were scored as follows: 0, no lesions present; 1, mild inflammatory changes in the colon or cecum or both, colitis with no evidence of necrosis, feces usually soft or loose, cecal or colonic walls or both slightly thickened; 2, cecal and colonic walls thickened, edematous and with ecchymotic hemorrhages or focal caseous necrosis or both, feces generally loose or watery, mesenteric lymph nodes with peripheral hyperemia and enlargement, splenomegaly sometimes present; 3, cecal and colonic walls thickened, edematous and with extensive hemorrhages or caseous necrosis or both, feces loose or watery, mesenteric lymph nodes with peripheral hyperemia and enlargement, splenomegaly sometimes present.

Statistical methods. Analysis of variance for a completely randomized design and Duncan's New Multiple Range Test (13) for pairwise comparisons of means were used. All pairwise comparisons of means of treatment groups were examined, and in the tables any two means that do not have a lettered superscript in common are statistically significantly different at the $P < 0.05$ level. The experimental units were the individual swine, unless otherwise indicated that the group of swine in the pen was used as the unit. On all percentage data in which the group of swine in the pen was used as the experimental unit, a double arcsine transformation was used and is defined as follows: arcsine $\sqrt{r/n + 1}$ + arcsine $\sqrt{(r + 1)/(n + 1)}$, where r = number in pen affected and n = total number in pen.

RESULTS

Noninoculated (noninfected) pigs were asymptomatic throughout the experiment, except for sporadic diarrhea (on days 1 through 13 of the experiment, 6 of the 12 pigs had diarrhea for 1, 2, or 3 days).

Initial clinical signs of salmonellosis in infected pigs consisted of a febrile response, anorexia, some vomiting, and loose or watery stools which appeared from 1 to 4 days PI. Diarrhea continued in some pigs up to the end of the experiment (day 26 PI). Deaths occurred between days 4 and 19 PI.

A significant febrile response occurred in all infected groups on day 1 PI (Table 2). On day 2 PI a significant dose response to treatment with cephamycin C was noted, in that successively lower temperatures were recorded as the level of medication increased. On day 3 PI the temperatures of the pigs medicated with 337.5 and 112.5 mg twice daily (b.i.d.) were significantly lower than the temperatures of the other infected groups. On day 5 PI the temperatures of the groups medicated with 337.5, 112.5, and 37.5 mg b.i.d. were significantly lower than the temperatures of the infected, nonmedicated group and were not significantly different from the temperatures of the noninfected, nonmedicated group. On days 7 through 14 PI there were no significant differences in temperatures among the infected, medicated groups and the noninfected, nonmedicated group.

Cephamycin C at 337.5 and 112.5 mg b.i.d. prevented diarrhea and mortality (Table 3). At

TABLE 2. *Febrile response of pigs infected with S. cholerae-suis and treated with cephamycin C intramuscularly or tetracycline orally for 10 days*[a]

Treatment (mg b.i.d.)	Febrile response (°C) on day postinoculation:					
	0	1	2	3	5	7
None (infected)	39.4^{A}	41.0^{A}	41.0^{A}	40.6^{A}	40.5^{A}	40.3^{A}
Tetracycline, 300	39.4^{A}	41.0^{A}	41.1^{A}	40.7^{A}	40.0^{B}	39.6^{B}
Cephamycin C, 12.5	$39.2^{A,B}$	41.0^{A}	41.0^{A}	40.8^{A}	40.0^{B}	39.6^{B}
Cephamycin C, 37.5	$39.3^{A,B}$	40.7^{A}	40.5^{B}	40.4^{A}	$39.7^{B,C}$	39.4^{B}
Cephamycin C, 112.5	39.0^{B}	40.8^{A}	40.0^{C}	39.8^{B}	39.4^{C}	39.2^{B}
Cephamycin C, 337.5	$39.2^{A,B}$	40.6^{A}	39.6^{D}	39.7^{B}	$39.6^{B,C}$	39.3^{B}
None (noninfected)	39.4^{A}	39.5^{B}	39.3^{D}	39.2^{C}	39.4^{C}	39.4^{B}

[a] Means for each day postinoculation with different-lettered superscripts differ significantly ($P < 0.05$).

TABLE 3. *Effects of treatment with cephamycin C intramuscularly or tetracycline orally for 10 days in pigs infected with S. cholerae-suis*[a]

Treatment (mg b.i.d.)	Mortality (%)[b]	Diarrhea (%)[c]			Weight gain (kg)		Feed efficiency[d]	Gut lesion index	
		S	D	S + D	S	D		S	D
None (infected)	83^{A}	54^{A}	71	$63^{A,B}$	$4.6^{B,C}$	−1.2	$-0.15^{B,C}$	2.0^{A}	2.9
Tetacycline, 300	$25^{B,C}$	45^{A}	84	54^{B}	2.6^{C}	−2.4	$0.13^{A,B,C}$	2.1^{A}	2.0
Cephamycin C, 12.5	$67^{A,B}$	50^{A}	90	75^{A}	1.9^{C}	−1.8	-0.26^{C}	1.3^{A}	3.0
Cephamycin C, 37.5	$25^{B,C}$	23^{B}	62	28^{C}	6.4^{B}	0.1	$0.32^{A,B}$	1.7^{A}	3.0
Cephamycin C, 112.5	0^{C}	2^{C}		2^{D}	10.8^{A}		0.46^{A}	0.3^{B}	
Cephamycin C, 337.5	0^{C}	$12^{B,C}$		$12^{C,D}$	11.9^{A}		$0.44^{A,B}$	0.1^{B}	
None (noninfected)	0^{C}	7^{C}		7^{D}	11.1^{A}		$0.43^{A,B}$	0.0^{B}	

[a] Means for each variable with different-lettered superscripts differ significantly ($P < 0.05$).

[b] Analyzed by using the group of swine in the pen as the unit.

[c] Percent diarrhea = 100% × total number postinoculation diarrhea days/total number postinoculation days observed. S, Surviving pigs (pigs that were alive on day 26 PI); D, dead pigs (pigs that died before day 26 PI). In the nonmedicated group, deaths occurred between days 5 and 11 PI; in the medicated groups deaths occurred between days 4 and 19 PI.

[d] Feed efficiency = weight gain/feed consumed. Analyzed by using the group of swine in the pen as the unit, with both surviving and dead pigs included.

37.5 mg b.i.d., cephamycin C significantly reduced both diarrhea and mortality and was comparable to tetracycline at 300 mg b.i.d. orally. The 12.5-mg b.i.d. level of cephamycin C was not effective in treating this salmonellosis.

The weight gains and feed efficiencies of the pigs treated with cephamycin C at 337.5 and 112.5 mg b.i.d. and the noninfected, nonmedicated control pigs were equivalent (Table 3). The pigs treated with these two highest levels of cephamycin C had essentially no gut lesions at necropsy.

All fecal samples from the pigs before inoculation and from the noninfected, nonmedicated control pigs throughout the study were negative for *Salmonella* spp. In the counts at 2 days PI, *S. cholerae-suis* was detected at a concentration ≥1,000 colony-forming units per g of feces in some pigs in all infected groups (Table 4). The groups treated with cephamycin C at 337.5 and 112.5 mg b.i.d. had significantly fewer pigs with counts ≥1,000 colony-forming units per g of feces, and none of the pigs in these groups were shedding *S. cholera-suis* in their feces from day 7 PI to the end of the experiment (day 26 PI).

At necropsy *S. cholerae-suis* was isolated from the tissues of 11 of 12 (89 to 95%) infected, nonmedicated pigs (Table 5). The groups medicated with cephamycin C at 337.5 and 112.5 mg b.i.d. had significantly fewer isolations from each of the four tissue samples (except for the ileocecal lymph node in the 112.5-mg b.i.d. group) than the infected, nonmedicated group, and the number of isolations from each of the tissues was not statistically significantly different from the completely negative tissues of the pigs in the noninfected, nonmedicated group.

DISCUSSION

A severe septicemic salmonellosis was induced in the pigs. The clinical and pathological features

TABLE 4. *Isolation of S. cholerae-suis from feces of live pigs*[a]

Treatment (mg b.i.d.)[b]	Day 2 PI		% Pigs positive on 1 or more of days 7, 14, and 21 PI[c]
	% Pigs with $\geq 10^3$ CFU/g feces[c]	Range of CFU/g of feces	
None (infected)	89^{A}	2.0×10^3 to 1.7×10^6	93^{A}
Tetracycline, 300	$33^{B,C,D}$	2.0×10^3 to 3.2×10^5	82^{A}
Cephamycin C, 12.5	$75^{A,B}$	6.0×10^3 to 6.3×10^5	$54^{A,B}$
Cephamycin C, 37.5	$50^{A,B,C}$	2.0×10^3 to 1.2×10^5	28^{B}
Cephamycin C, 112.5	$12^{C,D}$	4.0×10^3 to 3.6×10^4	0^{C}
Cephamycin C, 337.5	$25^{C,D}$	1.0×10^3 to 1.0×10^4	0^{C}
None (noninfected)	0^{D}		0^{C}

[a] Means for each variable with different-lettered superscripts differ significantly ($P < 0.05$).
[b] Tetracycline administered orally, cephamycin C administered intramuscularly.
[c] Analyzed by using the group of swine in the pen as the unit. CFU, Colony-forming units.

TABLE 5. *Isolation of S. cholerae-suis from tissues of pigs at necropsy*[a,b]

Treatment (mg b.i.d.)[c]	% Positive *S. choleraeusius* isolations from these samples[d]			
	Liver	Spleen	Ileocecal lymph node	Colon
None (infected)	89^{A}	95^{A}	95^{A}	89^{A}
Tetracycline, 300	$33^{A,B}$	$25^{B,C}$	$68^{A,B}$	33^{B}
Cephamycin C, 12.5	$50^{A,B}$	41^{B}	$75^{A,B}$	95^{A}
Cephamycin C, 37.5	18^{B}	$12^{B,C}$	$33^{A,B,C}$	25^{B}
Cephamycin C, 112.5	17^{B}	$17^{B,C}$	$18^{A,B,C}$	0^{C}
Cephamycin C, 337.5	0^{B}	0^{C}	$12^{B,C}$	0^{C}
None (noninfected)	0^{B}	0^{C}	0^{C}	0^{C}

[a] On day of death or on day 26 PI.
[b] Means for each variable with different-lettered superscripts differ significantly ($P < 0.05$).
[c] Tetracycline administered orally, cephamycin C administered intramuscularly.
[d] Analyzed by using the group of swine in the pen as the unit.

of the disease resembled those found in natural outbreaks and those described for experimentally induced *S. cholerae-suis* disease (4, 5, 9, 12). The initial clinical signs were pyrexia with anorexia and some vomiting, then diarrhea, and eventually death in nonmedicated pigs. On necropsy the most apparent gross lesions were found in the large intestine, where the walls of the cecum and colon were thickened and the mucous membrane was necrotic. The challenge *S. cholerae-suis* was routinely recovered at necropsy from the tissues of nonmedicated pigs.

Most of the antibiotics, nitrofurans, and sulfonamides used in animal health have been reported to be effective alone or in combination in prevention or treatment or both of swine salmonellosis under field or experimental conditions (2, 9–11). To rank these antibacterial agents for efficacy is difficult because of the varying severity of the disease conditions under which they were evaluated. In one study (11), wherein severity of disease was comparable to that in our study, infected, nonmedicated pigs had 80% mortality, whereas pigs medicated with nitrofurazone in water had 40% mortality, pigs medicated with furazolidone in feed had 60% mortality, and pigs medicated with chlortetracycline, sulfamethazine, plus penicillin in feed had 50% mortality. Furthermore, the average daily weight gain of each medicated group of pigs was approximately one half that of the noninfected, nonmedicated group. Comparison of the results reported for this study (11) with the observations made in our study is instructive. Cephamycin C prevented mortality and maintained the weight gain of infected, medicated pigs so that it was equivalent to the weight gain of the noninfected, nonmedicated control pigs. Such advantage shows that cephamycin C is a highly effective treatment for swine salmonellosis.

Recent studies have shown cephamycin C to be highly effective in treating inducing enterotoxigenic colibacillosis (scours) in both calves and piglets (6). In addition, in vitro studies (unpublished data) with multiply resistant enteropathogenic *Escherichia coli* and *Salmonella* spp. have shown no resistance to this drug.

These facts, combined with its resistance to degradation by β-lactamases, make cephamycin C a potentially useful drug for treatment of salmonellosis and colibacillosis in livestock industries.

Acknowledgment

We thank the Biometrics Unit of the Merck Institute for Therapeutic Research for performing the statistical analyses.

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