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Effect of Feeding Chlortetracycline on the Reservoir of *Salmonella typhimurium* in Experimentally Infected Swine

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Swine were fed either a diet containing 110 mg of chlortetracycline (CTC) per kg (100 g/ton) or a control diet and were inoculated orally with *Salmonella typhimurium* that was either susceptible or resistant to CTC. The quantity, duration, and prevalence of fecal elimination of *S. typhimurium*, as well as the effect of CTC on the transmission of *S. typhimurium* from infected to uninfected swine, were determined. When animals were infected with CTC-resistant *S. typhimurium*, CTC increased the quantity ($P < 0.05$), duration ($P < 0.05$), and prevalence ($P < 0.01$) of fecal shedding, the transmission from infected to uninfected swine, and the recovery of the infecting organism at necropsy. When animals were infected with CTC-susceptible *S. typhimurium*, CTC reduced the quantity (between 7 and 10 days postinfection) ($P < 0.01$), duration ($P < 0.05$), and prevalence ($P < 0.05$) of fecal shedding, the transmission from infected to uninfected swine, and the recovery of the infecting organism at necropsy. Resistance to tetracycline was transferred in vivo to 4 and 6% of the susceptible infecting *S. typhimurium* recovered from the untreated and treated groups, respectively. The increased reservoir of *S. typhimurium* and the transfer of resistance to susceptible *S. typhimurium* have implications for both animal and public health.

Widespread infections of *Salmonella* spp. occur in humans and animals (10, 16), with contaminated foods of animal origin as a major source of salmonellae for humans (3). There are reservoirs of salmonella in animals and animal feeds, the environment, and foods, as well as human carriers (3, 21). These could all be important in the perpetuation of salmonella infections.

The safety and efficacy of the use of antibiotics in animal feeds were reviewed by a task force for the Food and Drug Administration (21). As a result of that study, the Commissioner of the Food and Drug Administration concluded "that a significant increase in salmonella organisms in animals would constitute an increased hazard to human health" (7). One concern is that the feeding of subtherapeutic levels of antibiotics to animals should not result in an increased reservoir of salmonella or an increase in the occurrence of drug-resistant salmonella.

In a study in which swine were experimentally infected with a strain of *S. typhimurium* that was susceptible to chlortetracycline (CTC), the feeding of CTC at 220.5 g/metric ton of diet reduced the quantity of *Salmonella* in the feces, but the difference was statistically significant on only 1 day of testing (9). CTC also reduced the number of tissues that were positive for *Salmonella* at the end of the 30-day experiment. In another study, in which swine were experimentally infected with a strain of *S. typhimurium* that was susceptible to oxytetracycline, the feeding of oxytetracycline at 150 g/ton of diet (approximately 165 mg/kg of diet) did not significantly alter the quantity of *Salmonella* in the feces (5). It did, however, reduce (but not significantly) the number of positive samples. Neither of the above studies included a strain of *S. typhimurium* that was resistant to the drug being fed. In other studies, in which chickens or calves were experimentally infected with *S. typhimurium* that were resistant to the antibiotic being fed, the shedding of *Salmonella* in the feces was increased by the antibiotic being fed (20; B. P. Dey, D. C. Blenden, G. C. Burton, H. D. Mercer, and R. K. Tsutakawa, J. Am. Vet. Med. Assoc. 1975, Abstr. no. 77, p. 860).

This study was undertaken to determine

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whether feeding CTC to swine would cause an increase in the quantity, duration, or prevalence of elimination of *S. typhimurium* in the feces or an increased transfer of *S. typhimurium* from infected to uninfected swine if they were experimentally infected with a strain of *S. typhimurium* that was resistant to CTC. A second study was also conducted to study the same effects if swine were experimentally infected with a strain of *S. typhimurium* that was susceptible to CTC.

MATERIALS AND METHODS

Animal groups and their maintenance. Purebred Duroc swine (experiment I) or crossbred swine (experiment II) weighing between 8 and 18 kg were randomly allotted to experimental groups as shown in Table 1. Since group E-1 was not included in experiment I, 29 swine were used in that experiment, whereas 32 swine were used in experiment II. Except for animals that were placed in groups for purposes of contact, groups C-2 and T-2 (Table 1), animals were maintained in separate cages with no animal-to-animal contact. Each cage for groups C-1, T-1, E-1, and E-2 was placed in a separate pen so that there was no cage-to-cage contact. There was an aisle between all medicated and nonmedicated animals, except for groups C-2 and T-2.

The cages had slotted floors with trays underneath to catch waste material. The trays were removed from the cages for cleaning to avoid contact with the animal or its immediate surroundings. Pens for animals in groups C-2 and T-2 had concrete floors and were completely isolated from each other. Each of these pens was cleaned with separate cleaning instruments to avoid cross-contamination.

Personnel who collected samples and handled the animals wore gloves that were either changed or disinfected between handling each animal. Thermometers were thoroughly disinfected between use in each animal.

Animals were fed a diet containing 16% protein, prepared from cornmeal, soybean meal, alfalfa meal, and vitamin and mineral supplements. Feed and water were provided ad libitum. CTC was added to the diet at 110 mg/kg of feed for groups that were medicated. Medicated diet was fed to groups T-1, E-2, and T-3 from 5 days before experimentally infecting groups C-1 and T-1 with *S. typhimurium* until the end of the experiment.

Ten days after animals in groups C-1 and T-1 were infected, two animals from each group that had the highest and most consistent shedding rates were selected and placed in pens with C-2 and T-2 animals, respectively. The T-2 animals received medicated feed 5 days before the two T-1 animals were placed with them until the end of the experiment.

Groups E-1 and E-2 were environmental controls that were placed in building A to monitor the flow of *Salmonella* between animals. Animals in group E-1 were placed among C-1 animals, and E-2 animals were placed among T-1 animals. They were maintained, handled, sampled, and monitored in the same manner as the infected animals.

Organisms. The infecting *S. typhimurium* used in experiment I was resistant to CTC, streptomycin, ampicillin, and sulfonamide. Drug resistance was R-factor mediated. This strain of *S. typhimurium*, a member of serogroup B, was originally isolated from swine (obtained from the University of Illinois, Urbana). For experiment II, the infecting *S. typhimurium* (serogroup B) was resistant to nalidixic acid and susceptible to tetracycline, ampicillin, dihydrostreptomycin, cephalothin, sulfamethoxypyridazine, colistin, chloramphenicol, furazolidone, neomycin, and polymyxin B. This strain of *S. typhimurium* (S289-1) was obtained from the American Cyanamid Co., Princeton, N.J.

Inoculum. The inoculum was prepared by growing *S. typhimurium* on brilliant green agar (BGA) plates overnight at 37°C. Growth from each plate was washed off with approximately 10 ml of sterile saline and pooled. Organism counts were approximated by using

TABLE 1. *Experimental design*

Experimental group	No. of animals in group	Dosed with *S. typhimurium*	Treatment[a]	Maintenance of animals
C-1	7[b]	+[c]	−	Building A, individual cages, no animal-to-animal contact
E-1[d, e]	3	−	−	
T-1	7	+	+	
E-2[d]	3	−	+	
C-2	3	−	−	Pen 1, animal-to-animal contact
T-2	3	−	+	Pen 2, animal-to-animal contact
C-3	3	−	−	Building B, individual cages, no animal-to-animal contact
T-3	3	−	+	

[a] − denotes animals given control diet; + denotes animals given diet containing CTC.

[b] Two animals were moved from group C-1 to group C-2 and from group T-1 to group T-2 10 days after the animals in groups C-1 and T-1 were given *S. typhimurium*.

[c] + denotes animals orally dosed with a suspension of *S. typhimurium* as described in the text.

[d] Uninfected E-1 aimals were spread among C-1 animals and E-2 animals were spread among T-1 animals to monitor the flow of *S. typhimurium* among animals in building A.

[e] Group E-1 was not included in experiment I.

a Petroff-Hausser counter and were confirmed by plating logarithmic dilutions onto duplicate BGA plates.

Pooled inoculum was administered orally to each pig, using a 20-ml syringe with a 4-inch (ca. 10.16-cm) piece of plastic tubing attached to the end. With the aid of an oral speculum, the tip of the tubing was inserted into the posterior oral cavity, and the inoculum was discharged into the pharyngeal area. The number of organisms given to each animal in groups C-1 and T-1 was as follows: experiment I, 1.35×10^9; experiment II, 6.5×10^{10}.

Sample collection and processing. Early on each sampling day, each cage was cleaned and the animals were provided with fresh feed and water. Shortly thereafter, at least 10 g of fresh fecal droppings was collected from the cage tray with a wooden applicator stick and placed into sterile petri dishes. Animals housed together (groups C-2 and T-2) were sampled individually by rectally obtaining feces with a finger covered by a sterile surgical glove.

Three fecal samples from each animal were screened for *Salmonella* before the animals were placed in the experiments. For experiment I, fecal samples were collected daily for 8 days and then on days 10, 14, 16, 21, 23, 27, 30, 36, and 43 postinfection. For experiment II, fecal samples were collected daily for 11 days and then on days 13, 15, 17, 21, 24, 29, 36, 44, 50, and 57 postinfection. Except for samples collected for pretrial screening for *Salmonella*, sampling for groups C-2 and T-2 started on day 16 (experiment I) or day 11 (experiment II). All fecal samples were subjected to qualitative enrichment procedures for determining the presence of the infecting organism. A quantitative plate counting procedure was used to determine the concentration of infecting organisms in fecal samples obtained from experimentally infected animals during the first 10 days of experiment I and throughout experiment II.

Animals that had been experimentally infected were killed on either day 51 or day 52 postinfection (experiment I) or day 66 postinfection (experiment II). Liver, spleen, mesenteric lymph node, and colon content samples from each animal were examined by qualitative enrichment procedures.

Microbiological procedures. For qualitative assessment of *Salmonella*, fecal samples were swabbed directly onto BGA plates. One gram of fresh fecal material was placed in 9 ml of tetrathionate broth and incubated at 37°C overnight. The overnight enrichment was streaked onto BGA plates in duplicate and was subcultured in 9 ml of tetrathionate broth. The subculture and the initial tetrathionate broth enrichment were subsequently streaked onto BGA plates after overnight incubation at 37°C. Enrichment procedures were discontinued if positive results were obtained before the tests were completed. For enrichment of experiment II samples, 1 g of fecal material was placed in 9 ml of tetrathionate broth, incubated at 37°C for 48 h, and then streaked on a BGA plate. Nalidixic acid (50 μg/ml) was added to the BGA plates for experiment II. Tissue specimens were aseptically collected and minced. One gram of minced tissue or colon contents was then processed by qualitative procedures as described above for the fecal samples of each experiment.

To quantitate the *S. typhimurium*, 9 g of fresh feces was weighed into a disposable 200-ml plastic beaker, and 90 ml of phosphate-buffered saline (pH 7.2) was added (10^{-1} dilution). The beaker was closed with a snap-on lid, vigorously shaken by hand to disperse the feces, placed on a shaker apparatus, and gently agitated for 1 h at 4°C. Serial logarithmic dilutions through 10^{-7} were prepared by using phosphate-buffered saline (pH 7.2) as the diluent. A 0.1-ml portion of each dilution was spread on the surface of BGA plates in duplicate with bent glass rods. Nalidixic acid (50 μg/ml) was added to the BGA plates for experiment II. After incubation at 37°C for 18 to 20 h, colonies typical of salmonella were counted. When typical colonies were present in either the qualitative or the quantitative procedures, three colony-forming units were tested on triple sugar iron agar, and the colonies were subsequently serogrouped. Plate count results were rejected for samples when serogrouping was negative.

Serogrouping was conducted on all colonies that gave reactions on triple sugar iron agar slants that were typical of the infecting organism. One day before serogrouping, a loopful of growth from each triple sugar iron agar slant was streaked over the surface of a Trypticase soy agar slant, and the slants were incubated at 37°C for 24 h. The serogrouping suspension was prepared by washing growth from the Trypticase soy agar slants with approximately 0.5 ml of sterile saline. The suspension was transferred to a test tube (10 by 75 mm) and allowed to stand at room temperature for approximately 30 min. One drop of each cell suspension and 1 drop of group B antiserum (Difco Laboratories, Detroit, Mich.) were mixed on a glass plate and observed for agglutination.

Isolates (342) of the infecting *S. typhimurium*, selected from animals in which the infecting organism was recovered, were subjected to antimicrobial susceptibility tests. Susceptibility to ampicillin, streptomycin, cephalothin, sulfamethoxypyridazine, colistin, chloramphenicol, furazolidone, neomycin, polymyxin B, tetracycline, and nalidixic acid was tested by the method of Bauer et al. (2). Transferability of resistance was tested by the method of Schroeder et al. (19), using *Escherichia coli* K-12 (rifampin resistant) as the recipient. Five media were used in the test: MacConkey agar; MacConkey agar plus rifampin (20 μg/ml); MacConkey agar plus rifampin (20 μg/ml) and tetracycline (4 μg/ml); MacConkey agar plus rifampin (20 μg/ml) and streptomycin (25 μg/ml); MacConkey agar plus rifampin (20 μg/ml), ampicillin (10 μg/ml), and dicloxacillin (10 μg/ml). Recipients were tested by the method of Bauer et al. (2) to confirm the transfer of resistance.

Samples of control feed were examined for penicillin, streptomycin, neomycin, and CTC activity (12). Feed with added CTC was also assayed to confirm the CTC level (12). Feed was examined for *Salmonella* spp. by the enrichment procedures used for fecal material.

Statistical procedures. Data obtained from both experiments were statistically analyzed. The number of infecting organisms shed was subjected to an analysis of variance. Quantitative data were transformed by using natural logarithms, where zero was inter-

preted as 1 organism per g of feces. The value of 100 organisms per g of feces was assigned to samples that were negative by direct plating methods but positive by enrichment procedures. The duration of elimination of the infecting organism was determined by using regression analysis to estimate the time and the rate for each animal. The model assumed that the number of organisms (after the data were transformed to logarithms) would decrease linearly to a constant value and then remain at that level. The rate of decrease of elimination of the infecting organism was estimated as the slope of the regression line. The constant level was chosen to be 100 organisms per g of feces. A two-sided Wilcoxon test was conducted to determine whether the rate of decrease was different for each group (11).

Qualitative data were analyzed by comparing proportions of animals that were positive for the infecting organism by the chi-square test (14). Only data from animals that were experimentally infected were considered.

RESULTS

Assay of the feed supplemented with CTC indicated that a CTC level of 112 to 119 mg/kg (102 to 108 g/ton) was present. One of the three samples of control feed used in experiment I contained 4.2 mg of CTC activity per kg of feed. Two of the four control feed samples used in experiment I were positive for penicillin activity (average of 0.69 mg of penicillin per kg of feed). No salmonella organisms were recovered from the feed.

Clinical signs in animals that were experimentally infected included mild to profuse bloody diarrhea, slight to no rise in body temperature, decreased feed consumption, and various degrees of depression. During both experiments, the mean rectal temperatures remained between 100 and 104°F (ca. 37.8 and 40°C) for both study groups.

There were 20 animal days (occurrence in one animal on 1 day equals 1 animal day) of diarrhea for group T-1-I (I = experiment I) versus 8 for Group C-1-I. There were 24 and 11 animal days of diarrhea for groups C-1-II and T-1-II, respectively. Three animals died in group C-1-II, one each on days 7, 11, and 31 postinfection. Two animals in group T-1-II died 6 days after infection; the infecting organism was recovered from the liver, spleen, mesenteric lymph nodes, and colon contents of one animal and from the colon contents of the other animal. For animals in C-1-II, the infecting organism was recovered from the spleen, liver, mesenteric lymph nodes, and colon contents of the animal that died on day 7; from the liver, mesenteric lymmh nodes, and colon contents of the animal that died on day 11; and from the mesenteric lymph nodes and colon contents of the animal that died on day 31. The primary lesions were in the lungs, with lesser lesions in the lower intestines. Cold weather and high humidity had stressed the animals. Death was attributed to pneumonia complicated by the stress of experimental *S. typhimurium* infection.

Salmonella spp. were not recovered from any animals before the experiments started. Throughout both experiments, *Salmonella* spp. were not recovered from groups E-1, E-2, C-3, and T-3. The absence of *Salmonella* spp. in groups E-1 and E-2 indicates that there was no flow of the infecting organism among groups C-1 and T-1.

The recovery of the infecting organism from groups C-1-I and T-1-I is shown in Table 2. The number of organisms in both groups showed a decreasing trend with time. A significantly ($P < 0.05$) greater quantity of infecting organisms was eliminated by animals in group T-1-I. Most of the animals sporadically shed the infecting organism throughout the late part of the experiment. The pattern varied considerably according to the individual animal. The last positive sample for animal 6 was on day 14, and animal 5 had one positive sample out of the last eight. Samples from animal 8 were negative after day 8, but the remainder of the animals in T-1-I were positive on many of the last 8 sampling days. Although the duration of shedding for group T-1-I was significantly longer ($P < 0.05$) than that for the control group, the rates of decrease of the infecting organism for the two groups were not significantly different. The prevalence of shedding was measured by the proportion of positive samples. A significantly ($P < 0.01$) greater number of samples was positive for the infecting organism for group T-1-I as determined by the chi-square test.

Results of both qualitative and quantitative recovery of the infecting organism from groups C-1-II and T-1-II show a decreasing trend in quantity as the experiment progressed (Table 3). A significantly ($P < 0.01$) greater number of infecting organisms was eliminated during day 7 through day 10 by group C-1-II. There were no significant differences in days 1 through 5 and days 11 through 29. Because of animal deaths, data were analyzed for periods in which the number of animals remained constant. The duration of shedding for the two groups was not significantly different when animals that died were included in the analysis. When these animals were excluded, group C-1-II had significantly ($P < 0.05$) longer shedding times. The prevalence of shedding was significantly ($P < 0.05$) greater in group C-1-II.

Positive samples occurred on every sampling day and in 15 of the 21 total samples for group T-2-I, compared with 2 of the 21 positive samples

TABLE 2. *Recovery of S. typhimurium from swine after experimental infection with a strain of S. typhimurium resistant to CTC*

Pig no.	Colony-forming units of *S. typhimurium* per g of feces									Qualitative recovery of *S. typhimurium*								Total samples positive/ total samples collected
	1[a]	2	3	4	5	6	7	8	10	14	16	21	23	27	30	36	43	
Group C-1-I (untreated)																		
1	7.0×10^3	3.5×10^6	3.8×10^4	$<10^{2}$[b]	4.3×10^5	0[c]	$<10^2$	1.5×10^3	0	0	+[d]	0	0	+	+	+	0	
2	3.0×10^4	1.3×10^5	3.9×10^4	4.0×10^3	$<10^2$	2.1×10^5	$<10^2$	$<10^2$	0	+	0	0	0	+	0	+	0	
3[e]	4.0×10^5	1.5×10^3	3.0×10^3	0	1.4×10^4	1.5×10^4	1.8×10^5	1.0×10^4	$<10^2$	+	+	+	0	0	0	+	0	
4	4.0×10^5	2.1×10^5	$<10^2$	0[f]	$<10^2$	$<10^2$	1.7×10^3	1.1×10^2	2.3×10^3	0	0	0	+	+	0	+	+	
5[e]	4.9×10^5	9.0×10^4	$<10^2$	2.5×10^3	7.9×10^4[f]	6.9×10^4[f]	3.0×10^4[f]	1.9×10^3[f]	$<10^2$	0	0	0	0	0	+	0	0	
6	1.5×10^5	6.5×10^5	0	0	3.0×10^3	$<10^2$	$<10^2$	0	$<10^2$	+	0	0	0	0	0	0	0	
7	5.0×10^6	7.8×10^5	2.5×10^6[f]	1.5×10^4[f]	6.1×10^3[f]	$<10^2$[f]	$<10^2$	0	1.5×10^3	+	0	+	+	+	+	0	0	
Geometric mean	2.0×10^5	1.8×10^5	2.0×10^3	7.6×10^1	5.1×10^3	8.0×10^2	9.9×10^2	1.6×10^2	6.2×10^1									83/119
Group T-1-I (treated)[g]																		
8	4.5×10^4	$<10^2$	1.5×10^3[f]	1.3×10^5	$<10^2$	6.6×10^4	$<10^2$	1.8×10^4	0	0	0	0	0	0	0	0	0	
9	1.0×10^7	6.0×10^4	6.5×10^3	1.6×10^4	1.7×10^4	$<10^2$	$<10^2$	0	$<10^2$	+	0	+	+	0	+	+	+	
10[e]	7.8×10^5	4.5×10^3	$<10^2$	0	$<10^2$	8.2×10^5	3.3×10^6	5.8×10^5	$<10^2$[f]	0	+	+	+	0	0	0	+	
11	$<10^2$	4.1×10^4	6.8×10^5[f]	2.1×10^6	1.1×10^6[f]	$<10^2$	8.0×10^2	2.7×10^4	$<10^2$	+	0	0	+	0	+	+	+	
12[e]	4.0×10^7	1.8×10^6[f]	$<10^2$	2.0×10^4[f]	2.1×10^7[f]	3.8×10^5[f]	2.5×10^5	1.3×10^4[f]	7.8×10^5	+	+	0	+	+	0	+	+	
13	1.7×10^6	5.0×10^6	2.9×10^5	2.0×10^4[f]	6.9×10^3[f]	9.9×10^3[f]	1.4×10^4	1.0×10^4	8.2×10^3	+	+	+	+	+	+	+	0	
14	2.0×10^6	1.4×10^4[f]	4.8×10^4[f]	1.0×10^5[f]	4.9×10^4[f]	1.0×10^3[f]	2.5×10^3[f]	1.8×10^3[f]	1.0×10^4[f]	+	0	+	+	+	+	+	0	
Geometric mean	4.7×10^5	3.9×10^4	7.1×10^3	1.5×10^4	2.0×10^4	8.0×10^3	5.8×10^3	4.9×10^3	6.8×10^2									101/119

[a] Days post-inoculation.
[b] Positive after enrichment; represented as 1.0×10^2 for determination of geometric mean.
[c] Negative after enrichment.
[d] Positive after enrichment; only enrichment procedures were used after day 10.
[e] Animals moved from their individual cages and placed in either pen 1 or pen 2 with animals in group C-2-I or group T-2-I at 10 days post-inoculation.
[f] Clinical diarrhea present.
[g] Animals received diet containing CTC at 110 mg/kg of diet.

for group C-2-I. Both of the latter positive samples occurred 17 days after contact with the two positive shedder animals from group C-1-I that were placed with them. In contrast to experiment I, 22 of 33 samples for group C-2-II were positive, and positive samples occurred on every sampling day. For group T-2-II, 8 of 33 samples were positive, and no positive samples were found on 6 of 11 sampling days. The first positive sample for group T-2-II was on day 5 of contact with the two shedder animals from group T-1-II.

After the animals were killed, the colon contents of one animal in group C-1-I was positive. For T-1-I animals, the positive samples included colon contents from five animals plus a mesenteric lymph node from one of these animals and a liver sample in one animal in which all other necropsy samples were negative. In contrast, positive samples for C-1-II animals included colon contents from the four remaining animals plus a mesenteric lymph node from one animal, whereas the colon contents from one animal in group T-1-II was positive. The colon contents of one animal and the mesenteric lymph node from another were positive in group T-2-I. Colon contents from one animal in group C-2-II was positive.

The infecting organism in experiment II was susceptible to CTC at the time of administration to the animals. Results of susceptibility testing of the infecting organism recovered throughout the study are given in Table 4. Six percent of the isolates obtained from group T-1-II were resistant to tetracycline, compared with 4% from group C-1-II. A total of 19 and 20% of the *S. typhimurium* isolates recovered from groups T-1-II and C-1-II, respectively, were resistant to at least one drug in the susceptibility test. Resistance to a single drug, most frequently streptomycin, was most common, followed by resistance to two antibiotics. Resistance transfer occurred for both single and multiple resistances. Transfer of resistance was detected in 14 of the 17 resistant isolates of *S. typhimurium* from group C-1-II that were tested. Five of seven isolates from group C-2-II transferred resistance. Transfer of resistance occurred in 11 of 19 isolates from group T-1-II. One isolate from group T-2-II was tested, and transfer did not occur.

DISCUSSION

The possibility that the antibiotic residues found in some of the control feed may have influenced the results should be considered. If the penicillin activity (~0.69 mg/kg of feed) in experiment I or the CTC activity (4.2 mg/kg of feed) in experiment II did influence the infecting organism, the results for the untreated group should have been similar to those of the treated group; yet results in both experiments were significantly different. The influence of CTC or penicillin activities in some of the control feed is unknown; however, this activity was not sufficient to mask differences between treatment groups, and it probably had little influence on results. Drug absorption, stability characteristics, and dilution effects would all serve to reduce the effect of both penicillin and CTC.

Quantitative results were not given on a dry-weight-of-feces basis because the moisture content of fecal samples was not determined. Giving quantitative results on a dry-weight basis is considered the preferable procedure. Diarrhea occurred more often in animals in group T-1-I which shed a significantly greater quantity of *Salmonella* than did animals in group C-1-I. Likewise, diarrhea occurred more often in C-1-II animals which shed a significantly greater quantity of *Salmonella* than did T-1-II animals. Calculating the concentration of *Salmonella* on a dry-weight basis would have accentuated the differences in both experiments because this calculation will cause the concentration that results from wet weight to increase.

Results from experiment II, in which swine were experimentally infected with CTC-susceptible *S. typhimurium*, show that the feeding of CTC at a subtherapeutic level did not result in increased shedding but, in fact, resulted in decreased shedding. This confirms results from other studies in which either no effect or, perhaps, decreased shedding occurred when subtherapeutic levels of CTC or oxytetracycline were fed to chickens (5, 13), turkeys (18), and calves (5, 15), as well as swine (5, 9) that were all experimentally infected with a strain of *S. typhimurium* that was susceptible to the drug that was used.

Our two experiments show the importance of the susceptibility of the strain of *S. typhimurium* that is used to infect experimental swine when the effect of an antibiotic on salmonella shedding is studied. Contrasting results were obtained for every aspect of shedding studied. When the infecting organism was resistant to CTC, the use of CTC at 110 mg/kg of feed increased the quantity, duration, and prevalence of *Salmonella* shedding; yet, when animals were infected with a strain that was susceptible to CTC, the protective effect of CTC was shown by reducing the quantity, duration, and prevalence of shedding. Contrasting results were also obtained on the transmission of *Salmonella* from infected to uninfected swine. The use of CTC enhanced the transmission of *Salmonella* between animals and the time necessary for transmission when the infecting organism was resistant to CTC,

TABLE 3. *Recovery of S. typhimurium from swine after experimental infection with a strain susceptible to CTC*

Pig no.	Colony-forming units of *S. typhimurium* per g of feces										
	1[a]	2	3	4	5	6	7	8	9	10	11
Group C-1-II (untreated)											
101[b]	2.9×10^6	6×10^4	5.9×10^{6c}	2.7×10^{6c}	2.0×10^{7c}	8.0×10^{6c}	1.8×10^{6c}	1.1×10^{5c}	4.1×10^4	1.1×10^4	$<10^{2d}$
102	3.6×10^6	1.7×10^5	9.5×10^4	6.5×10^4	2.5×10^4	6.3×10^{4c}	4.3×10^4	8.0×10^3	1.0×10^3	1.0×10^3	3.5×10^3
103[b]	1.4×10^6	2.2×10^5	1.3×10^6	2×10^5	4.3×10^5	1.1×10^6	4.3×10^5	3.2×10^4	9.5×10^3	9.5×10^{4c}	3.5×10^4
104	3.5×10^3	7×10^4	1.4×10^6	3×10^5	4.5×10^{5c}	3.0×10^{4c}	4.2×10^{4c}	2.7×10^4	2.5×10^4	6.7×10^{4c}	3.2×10^4
105	$<10^2$	2×10^3	2×10^3	1.5×10^3	$<10^2$	$<10^2$	3.0×10^3	$<10^2$	1.0×10^3	9.5×10^{3c}	$<10^2$
106	$<10^2$	7.7×10^{6c}	1.5×10^{6c}	6.1×10^{4c}	1.1×10^4	3.3×10^4					
107	5.3×10^5	2.5×10^6	6.5×10^{5c}	3.2×10^{4c}	6.5×10^{4c}	2.5×10^{5c}	4.2×10^{5c}	9.6×10^5	1.2×10^5	1.2×10^4	
Geometric mean	2.2×10^4	1.3×10^5	2.1×10^5	8.4×10^4	6.8×10^4	7.5×10^4	1.1×10^5	2.0×10^4	1.0×10^4	1.4×10^4	2.1×10^3
Group T-1-II (treated)[e]											
108	1.6×10^4	3.5×10^3	$<10^2$	$<10^2$	7.0×10^3	1.5×10^3	0	1.0×10^3	$<10^2$	$<10^2$	$<10^2$
109	1.8×10^7	3.0×10^4	$<10^2$	1.8×10^4	6.0×10^4	$<10^2$	$<10^2$	$<10^2$	$<10^2$	7.0×10^3	1.0×10^3
110	1.4×10^4	$<10^2$	1.0×10^3	$<10^2$	6.0×10^5	3.0×10^{3c}	1.0×10^{3c}	6.0×10^3	$<10^2$	1.0×10^3	$<10^2$
111	6.1×10^5	4.7×10^{4c}	1.6×10^{5c}	3.9×10^{5c}	1.0×10^3						
112[b]	1.4×10^6	3.2×10^{4c}	1.0×10^{4c}	$<10^2$	1.0×10^3	2.0×10^3	$<10^2$	1.0×10^3	$<10^2$	2.5×10^3	5.0×10^2
113	2.3×10^6	8.0×10^3	9.0×10^3	1.0×10^3	1.1×10^{5c}	6.0×10^{3c}	$<10^2$	$<10^2$	$<10^2$	4.0×10^3	1.5×10^{3c}
114	$<10^2$	2.5×10^{5c}	4.2×10^5	4.6×10^5	$<10^2$						
Geometric mean	2.2×10^4	1.2×10^4	4.8×10^3	3.2×10^3	8.3×10^3	1.4×10^3	6.3×10^1	5.7×10^2	1.0×10^2	1.5×10^3	3.8×10^2

TABLE 3—*Continued*

Pig no.	Colony-forming units of *S. typhimurium* per g of feces										Total samples positive/ total samples collected
	13	15	17	21	24	29	36	44	50	57	
Group C-1-II (untreated)											
101[b]	2.5×10^3	1.0×10^4	2.5×10^3	1.5×10^2	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	
102	$<10^2$	$<10^2$	$<10^2$	6.0×10^3	5.0×10^2	$<10^2$	1.0×10^3	$<10^2$	5.0×10^2	$<10^2$	
103[c]	7.5×10^3	$<10^2$	7.0×10^3	3.5×10^3	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	
104	3.5×10^{5c}	3.7×10^4	8.5×10^3	3.4×10^4	2.3×10^{4c}	3.3×10^{5c}					
105	$<10^2$	$<10^2$	$<10^2$	$<10^{2c}$	$<10^2$	$<10^2$	0	0	$<10^2$	1.5×10^3	
106											
107											
Geometric mean	2.3×10^3	8.2×10^2	1.1×10^3	1.6×10^3	4.1×10^2	5.1×10^2	5.6×10^1	3.2×10^1	1.5×10^2	2.0×10^2	115/117
Group T-1-II (treated)[e]											
108	5.0×10^2	1.0×10^3	$<10^2$	$<10^2$	$<10^2$	1.0×10^3	2.9×10^4	$<10^2$	$<10^2$	0	
109	2.0×10^3	3.5×10^3	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	1.5×10^3	4.0×10^3	1.1×10^4	
110	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	0	$<10^2$	$<10^2$	0	0	
111											
112[b]	$<10^2$	$<10^2$	5×10^2	$<10^2$	$<10^2$	$<10^2$	0	0	0	$<10^2$	
113	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	0	0	0	
114											
Geometric mean	2.5×10^2	3.2×10^2	1.4×10^2	1.0×10^2	1.0×10^2	6.3×10^1	1.2×10^2	2.7×10^1	1.3×10^1	1.6×10^1	104/115

[a] Days post-inoculation.
[b] Animals moved from their individual cages and placed in either pen 1 or pen 2 with animals in group C-2-II or group T-2-II at 10 days post-inoculation.
[c] Clinical diarrhea present.
[d] Positive after enrichment; represented as 1.0×10^2 for determination of geometric mean.
[e] Animals received diet containing CTC at 110 mg/kg of diet.

TABLE 4. *Drug resistances in isolates of S. typhimurium recovered from swine that were experimentally infected with a nalidixic acid-resistant but otherwise drug-susceptible S. typhimurium (experiment II)*

Experimental group	CTC in feed (mg/kg)	No. of isolates with resistance to[a]:										Total no. of isolates tested
		Ap	Sm	Cf	Su	Cl	C	Fx	Nm	Tc	Nx[b]	
C-1-II (experimentally infected)	0	3	14	6	3	2	1	2	1	5	156	156
C-2-II (animals in contact with animals eliminating *S. typhimurium*)	0	1	5	0	0	1	0	0	1	2	20	20
T-1-II (experimentally infected)	110	3	15	1	0	2	1	1	3	9	158	158
T-2-II (animals in contact with animals eliminating *S. typhimurium*)	110	0	0	0	0	1	0	1	0	1	7	7

[a] Paper disk potency: Ap, ampicillin (10 μg); Sm, streptomycin (10 μg); Cf, cephalothin (30 μg); Su, sulfamethoxypyridazine (250 μg); Cl, colistin (10 μg); C, chloramphenicol (30 μg); Fx, furazolidone (100 μg); Nm, neomycin (30 μg); Tc, tetracycline (30 μg); Nx, nalidixic acid (30 μg).

[b] Value also corresponds to total number of isolates tested.

whereas it decreased the transmission and time necessary for transmission when the infecting strain was susceptible to CTC. Recovery of the infecting organism from animals at necropsy also showed the same pattern. More colon contents were positive after use of CTC when animals were infected with a CTC-resistant strain, and fewer were positive when a susceptible strain was used. The few tissue recoveries also corresponded in the same manner.

The *S. typhimurium* used in experiment II was resistant to nalidixic acid (nontransferable) and susceptible to the other antibiotics for which it was tested; yet resistance to nine antimicrobial drugs was found among the *S. typhimurium* organisms recovered from the experimental animals. This represents an increase in the reservoir of drug-resistant *Salmonella*. Because the resistance to streptomycin and tetracycline found in the *S. typhimurium* organisms was transferable, we conclude that the *S. typhimurium* organisms acquired much of their resistance by in vivo transfer.

Resistance transfer studies were conducted only to document that there was transferable resistance. There was an insufficient number of isolates tested from each group to draw any conclusions on the difference of transferability of resistance between test groups of animals.

The proportion of drug-resistant isolates of *S. typhimurium* was similar in both C-1-II and T-1-II animals. The rate of transfer of drug resistance in a study such as this may be related to a number of factors: the presence or absence of antibiotic (1, 6, 8), plasmid content of the donor and recipient populations (4), bioserotype of the recipient salmonella (17), and quantity of donor R^+ bacteria in the gut of the swine.

The animals in this experiment were not tested for the incidence of transferable drug resistance in their gut flora. We suspect, however, from testing other swine in this research facility that a high proportion of drug-resistant, gram-negative enteric bacteria existed in the swine used in these experiments before they received CTC. If this were the case, the CTC would have had little or no chance of increasing the proportion of drug-resistant donor bacteria in the gut. Thus, the rate of transfer of drug resistance in this experiment was probably due more to the incidence of donors than to the presence of CTC. The results also indicate that CTC did not select for resistant salmonella once they had become resistant.

Results of experiment II show that feeding CTC at a subtherapeutic level to swine can reduce the salmonella reservoir in swine if they are infected with a susceptible strain. The results also demonstrate that susceptible salmonella can become resistant when there are donors. This should be evaluated in view of results obtained from experiment I. As we have shown, feeding a subtherapeutic level of CTC to swine infected with CTC-resistant *S. typhimurium* can increase the salmonella reservoir.

Increasing the quantity of resistant *S. typhimurium* by either mechanism is undesirable. By increasing the quantity of salmonella in swine, the chances of perpetuating salmonellosis in both animals and humans are increased. Increasing the quantity of resistant salmonella reduces prospects of therapeutic success in the treatment of salmonellosis in animals or systemic salmonellosis in humans.

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