Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 10 *Swine Day (1968-2014)*

Article 690

1997

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EFFECTS OF TETRACYCLINE ON SHEDDING OF SUSCEPTIBLE AND RESISTANT *SALMONELLA* SPP. EXPERIMENTALLY INOCULATED INTO PIGS[1]

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Summary

The objective of this experiment was to study the influence of tetracycline on the transfer of antibiotic resistance in an *in vivo* swine model experimentally infected with antibiotic-resistant and antibiotic-susceptible *Salmonella* spp. Tetracycline reduced the amount and duration of shedding of tetracycline-susceptible *Salmonella*. However, tetracycline had no effect on shedding of resistant *Salmonella*. We also have evidence that resistance was transferred from the resistant to the susceptible strain of *Salmonella*.

(Key Words: *Salmonella typhimurium*, Antibiotics, Resistance.)

Introduction

Antibiotics are used commonly to treat infections in animals and humans and frequently are fed at subtherapeutic levels to food animals for growth promotion. These practices may be associated with the development of antibiotic resistance, but little information is available about the mechanism involved. Infections caused by antibiotic-resistant *Salmonella*, such as the multidrug-resistant *Salmonella typhimurium* Definitive Type 104 (DT 104), are increasing and have become a cause for public health concern. Our arsenal of effective antibiotic therapy is diminishing as resistance increases and the cost of developing new antibiotics escalates. Thus, it is urgent to study and better understand the acquisition of resistance factors so that antibiotics currently available can be used more effectively. Therefore, our objective was to study the influence of tetracycline on the transfer of antibiotic resistance in an *in vivo* swine model.

Procedures

Twenty pigs (10 barrows and 10 gilts) were assigned randomly to one of two groups 3 days prior to the start of the experiment. At the time of randomization, pigs were 35 d of age. Five barrows and five gilts were assigned to each group, tetracycline-treated and control. Results of randomization yielded a design balanced on treatment, gender, and body weight within gender.

All pigs had *ad libitum* access to control feed (without growth-promoting antibiotics) and nipple waterers for the 3 d acclimation period and were housed in pens in an environmentally controlled building (temperature ranged from 68°C to 78°C). On the d 0 of the experiment, the treatment group was switched to a diet containing chlortetracycline (200 g/ton of feed). The treatment group also was given 5 mg/lb BW of oxytetracycline IM on d 3, 4, 5, and d 17, 18, 19. The injections were alternated between the left and right sides of the pig.

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[1]Appreciation is expressed to the American Veterinary Medical Association Foundation for providing partial financial support. Appreciation also is expressed to Dave Steuver, Stacy Stoltenberg, and Mike Hornback for technical assistance.

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On d 0, just prior to inoculation, rectal swabs were collected from all pigs. On d 0 and 14, all pigs were inoculated intra-gastrically by oral catheter with a mixture of 10^9 *Salmonella typhimurium* (tetracycline susceptible) and 10^9 *Salmonella anatum* (tetracycline resistant). Pigs were observed daily for clinical signs of lethargy, inappetence, and diarrhea.

Rectal swabs also were collected on d 2, 4, 7, 9, and 11. Fecal samples were collected by rectal palpation on d 14, 16, 18, 21, 23, 25, 28, 30, 32, and 35. Samples were cultured according to standard microbial isolation techniques.

Any isolated *Salmonella* colonies were counted, and colony forming units (cfu) per ml were calculated. On all plates with suspected *Salmonella* spp., up to six individual colonies were selected and serotyped by agglutination procedures using *Salmonella* O Antiserum Groups E and B. All isolates then were streaked onto a brain-heart infusion (BHI) agar slant, cataloged, and refrigerated.

On d 35, all pigs were euthanized and necropsied. Using sterile technique, the following tissues were collected: spleen, liver, kidney, ileocecal lymph node, ileocecal wall, bone marrow, tonsils, and lung. Swab samples were obtained from the jejunum, ileum, cecum, ileocecal junction, colon, and spiral colon contents for culturing of *Salmonella* as previously described.

A subsampling of 163 isolates was checked for tetracycline sensitivity by the Sensititre Microbiology method. Isolates from the first and last day of *Salmonella* spp. shedding (1 or 2 isolates per pig per d depending on whether one or both *Salmonella* spp. strains were present) as well as the tissue swabs taken at necropsy were tested.

The pig was considered the experimental unit for all statistical analysis. A pig was considered to be shedding *Salmonella* spp. each day when at least one *Salmonella* spp. isolate was obtained. The effect of tetracycline on shedding rate was analyzed as a split plot design with the control or medicated group as the whole plot and day of sampling as the subplot.

Results

Few signs of clinical salmonellosis were observed. We failed to isolate any *Salmonella* spp. from the swabs obtained prior to inoculation. A total of 1,247 *Salmonella* spp. isolates was collected. These isolates were characterized as *S. anatum* (n=975) or *S. typhimurium* (n=272).

Medication by day interactions were not noted. *S. anatum* (tetracycline resistant) was shed in a greater number than was *S. typhimurium* (tetracycline susceptible; Table 1). *S. anatum* was shed in higher numbers immediately after both inoculations and then the number shed declined (Figure 1).

Table 1. Percentage of Samples from Which the Specified *Salmonella* sp. Was Isolated

Species Isolated	Control	Tetracycline	SE
S. typhimurium only[a]	10.0%	2.0%	2.7%
S. anatum only	37.3%	32.7%	5.1%
Both[b]	12.0%	2.0%	2.4%
None[b]	40.7%	63.3%	5.3%

[a]Control vs. tetracycline ($P<.06$).
[b]Control vs. tetracycline ($P<.01$).

A total of 112 isolates was extracted from the ileocecal lymph node, ileocecal wall, and tonsil tissues taken at necropsy. The isolates were characterized as *S. anatum* (n=94) or *S. typhimurium* (n=18) and further broken down into treatment groups. The medicated treatment group had 36 *S. anatum* and 6 *S. typhimurium* isolates. The control treatment group had 58 *S. anatum* and 12 *S. typhimurium* isolates.

Of the 163 fecal isolates tested for antibiotic resistance, 74 were *S. anatum* and 89 were *S. typhimurium*. Of the 74 *S. anatum*,

2 were susceptible and 72 were resistant to tetracycline. Results for *S. typhimurium* included 40 susceptible, 13 intermediate susceptible, and 36 resistant isolates. The medicated treatment group had 3 susceptible, 8 intermediate susceptible, and 9 resistant *S. typhimurium*. Ten susceptible, 32 intermediate susceptible, and 27 resistant *S. typhimurium* were isolated from the control treatment group.

Discussion

The overall pattern of shedding showed that *S. anatum* was shed by a greater number of pigs than was *S. typhimurium* (Figure 1). This was expected, because *S. anatum* was antibiotic resistant and *S. typhimurium* was antibiotic susceptible. The pattern was especially evident when shedding was compared by treatment group within *Salmonella sp.* (Figures 2 and 3). More nonmedicated pigs than medicated pigs shed *S. typhimurium*. When shedding of *S. anatum* was compared between treatment groups, no difference was detected (Table 1). Although no conclusions can be made about whether the competition between the two strains *in vivo* was influenced by the presence of a resistance factor, the strain with the R factor was detected in a greater number of samples irrespective of the presence of tetracycline.

In conclusion, tetracycline had an impact on the amount and duration of fecal shedding of *Salmonella typhimurium*. In contrast, tetracycline did not have an effect on shedding rates of *Salmonella anatum*. Because the low numbers of samples and pigs from which *S. typhimurium* was isolated in the medicated group, we were unable to determine if tetracycline had an influence on resistance transfer. Preliminary results indicate the possibility of transfer of tetracycline resistance occurring between *S. anatum* and *S. typhimurium*.

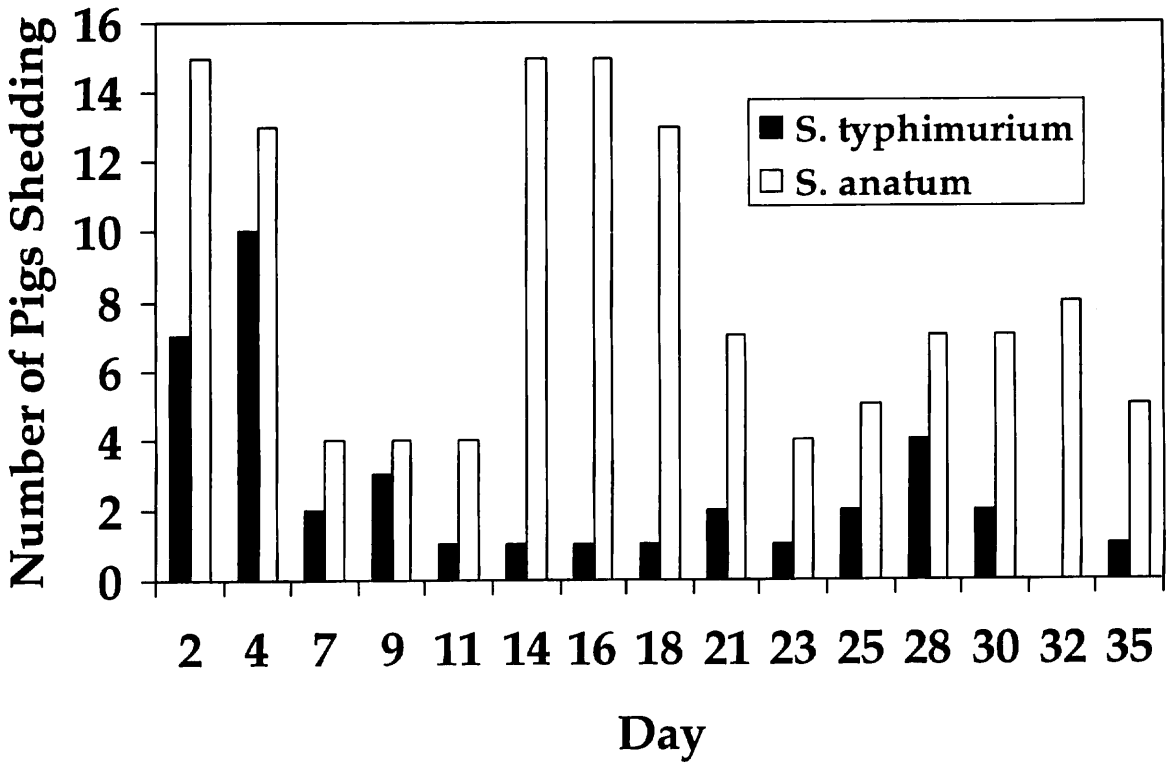

Figure 1. Pattern of *Salmonella* spp. Shedding over Time

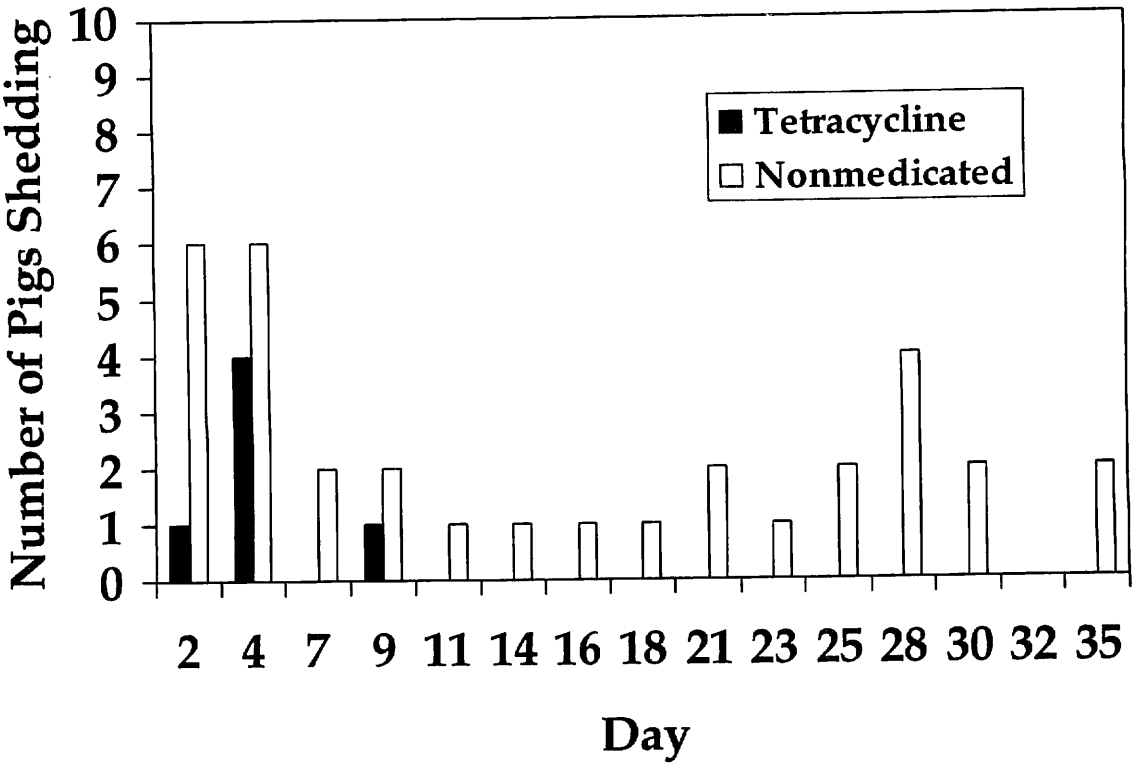

Figure 2. Influence of Tetracycline on Pattern of *Salmonella typhimurium* (Tetracycline Susceptible) Shedding over Time

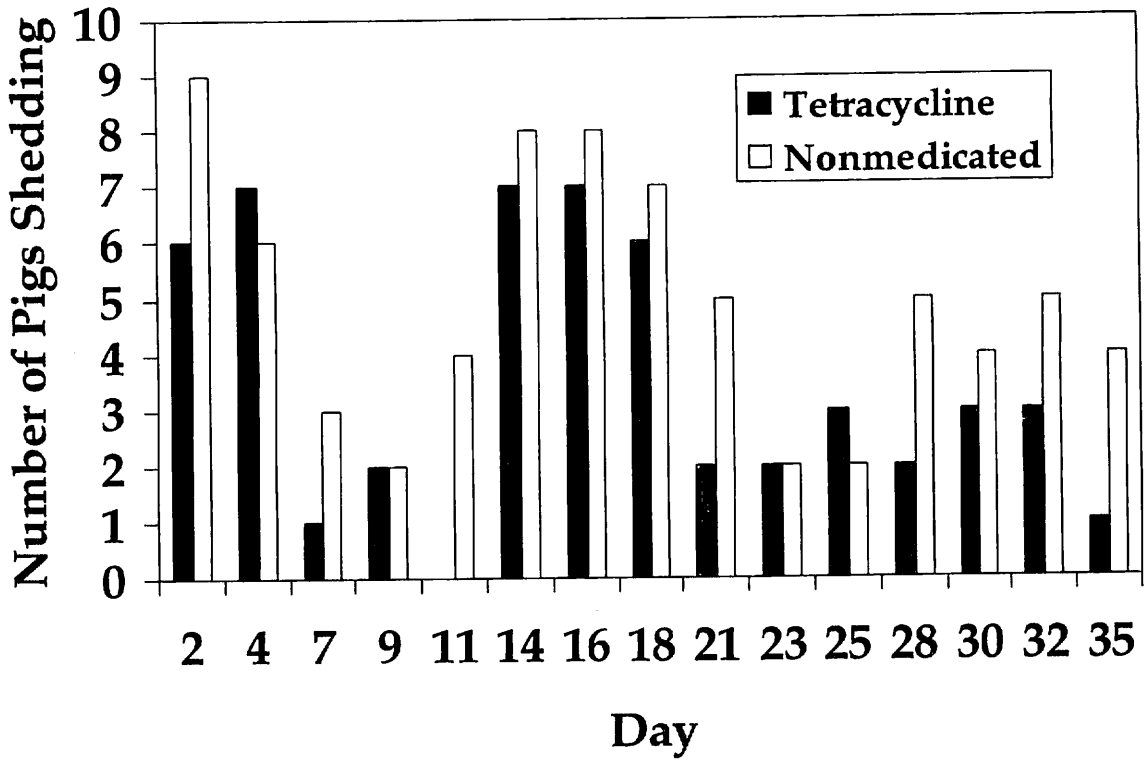

Figure 3. Influence of Tetracycline on Pattern of *Salmonella anatum* (Tetracycline Resistant) Shedding over Time