Kansas Agricultural Experiment Station Research Reports

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

Article 670

1996

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Abstract

The objective of this experiment was to determine if antibiotics used as feed additives and disease treatment for livestock affect duration of shedding and colonization of tissues with Salmonella typhimurium in pigs. No statistically significant difference was detected in duration or amount of shedding of S. typhimurium between pigs receiving antibiotics and control pigs. Antibiotics prevented colonization of tissues by S. typhimurium. The odds (OR= .02) of isolating S. typhimurium in at least one of four tissues examined were significantly less from pigs treated with antibiotics than from control pigs (two-tailed Fisher exact test, P= .009).; Swine Day, Manhattan, KS, November 21, 1996

Keywords

Swine day, 1996; Kansas Agricultural Experiment Station contribution; no. 97-142-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 772; Swine; Salmonella typhimurium; Nursery pigs; Antibiotics

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EFFECTS OF ANTIBIOTICS ON SHEDDING OF *SALMONELLA TYPHIMURIUM* IN EXPERIMENTALLY INOCULATED PIGS

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Summary

The objective of this experiment was to determine if antibiotics used as feed additives and disease treatment for livestock affect duration of shedding and colonization of tissues with *Salmonella typhimurium* in pigs. No statistically significant difference was detected in duration or amount of shedding of *S. typhimurium* between pigs receiving antibiotics and control pigs. Antibiotics prevented colonization of tissues by *S. typhimurium*. The odds (OR=.02) of isolating *S. typhimurium* in at least one of four tissues examined were significantly less from pigs treated with antibiotics than from control pigs (two-tailed Fisher exact test, $P=.009$).

(Key Words: *Salmonella typhimurium*, Nursery Pigs, Antibiotics.)

Introduction

Antibiotics are used to treat infections in animals and humans and are fed to food animals for growth promotion. These practices may be associated with development of bacteria resistant to antibiotics. Increased antibiotic resistance leads to increased risk to animal and human health. Infections caused by antibiotic-resistant *Salmonella* are increasing and are reasons for public health concern. Thus, obtaining a better understanding of *Salmonella* infections so that antibiotics currently available can be used more effectively is important.

The research described here was designed to determine the effect of subtherapeutic and therapeutic dosages of antibiotics on infection of pigs experimentally inoculated with *Salmonella typhimurium*.

Procedures

Subjects for the study were 18 3-week-old pigs (PIC, C15 x L326), one pig from each of 18 litters. Pigs were assigned randomly to either control or treatment groups, matched by initial body weight and gender. Results of randomization yielded a design balanced on treatment, gender, litter, and body weight within gender. Pigs were housed in a controlled isolation facility in concrete pens, one pig per pen. Each pig had access to feed and water ad libitum. Diets were prepared at the Kansas State University feed mill. After a 7-day acclimation period, all pigs were inoculated intragastrically with 4×10^9 CFU of *S. typhimurium* that was susceptible to the antibiotics used. After inoculation and until completion of the experiment, the treatment group received subtherapeutic levels of neomycin and tetracycline in feed (99.5 mg each /lb of feed) and tetracycline (4.5 mg/lb of body weight) in water. Ceftiofur was administered at therapeutic levels (15.1 mg/lb) to the treatment group on day 2 through day 6 postinoculation. The control group was not given antibiotics as feed or water additives or as treatments for disease.

All pigs were evaluated daily for clinical signs of disease; activity level (normal,

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lethargic, down); stool consistency (firm, soft, runny); and intake of feed and water. Body temperature was recorded, and rectal swab and blood samples were collected three times a week from each pig.

Rectal swabs were cultured for *S. typhimurium* using standard microbiological techniques. Amount of shedding was defined as the number of steps required to isolate *S. typhimurium*. If *S. typhimurium* could be isolated by direct swab, the amount of *S. typhimurium* in the sample was given a score of 1, indicating that *S. typhimurium* was easiest to isolate and, therefore, shed in the highest numbers. If *S. typhimurium* was isolated after a 24-h enrichment, the sample was given a score of 2. If further enrichment was required for isolation, the sample was given a score of 3.

At necropsy (day 25 postinoculation), swab samples of ileum, cecum, ileocecal junction, and colon were collected aseptically for bacterial isolation and identification. Four tissue sections (liver, spleen, lymph node, and colon) were cultured after extensive washing and mechanical disruption. Pigs that died before completion of the experiment were necropsied and sampled on the day of death.

Frequency data were tabulated and analyzed by use of EpiInfo. The SAS GLM procedure was used to analyze continuous data.

Results and Discussion

Two pigs from the control group died before the end of the experiment. One pig died of salmonellosis (day 8 postinoculation) and the other died from complications unrelated to the study (day 4). The pig that died on day 8 was necropsied and checked for colonization by swabbing several sites along the intestinal tract. *S. typhimurium* was isolated by direct plating at every portion of the tract that was sampled. No pigs from the treatment group died before the end of the experiment.

All pigs in both groups were shedding *S. typhimurium* on day 3 postinoculation. For the control group, 57% (4/7) of the pigs were shedding on the final day. For the treatment group, 22% (2/9) were shedding on day 25 (Table 1). Excluding the two pigs that died early and the four pigs that were shedding *S. typhimurium* on the final day of the study (and that might have continued to shed if the study was not terminated), the average duration of shedding was 21 days. Average duration of shedding for the treatment group, excluding the two pigs that were shedding *S. typhimurium* on the final day, was 18 days.

S. typhimurium was isolated from tissue swabs of all pigs in at least one site along the intestinal tract. *S. typhimurium* was isolated from four of the seven control pigs (57%) at every site sampled. *S. typhimurium* was isolated from only one of the nine treatment pigs (11%) at every sample site. For the control group, *S. typhimurium* was isolated most in the colon and spiral colon (Table 2). For the treatment group, *S. typhimurium* was isolated most frequently from the cecum, colon, and ileocecal junction. For both groups, *S. typhimurium* was isolated least from swab samples of the jejunum. *S. typhimurium* was isolated from swabs of the jejunum significantly less often from treatment pigs than from control pigs.

In the control group, 86% (6/7) of the pigs were colonized by *S. typhimurium* in at least one of the four tissues that were sampled (Table 1 and Figure 1). One pig from the group that received antibiotics (11%) was colonized by *S. typhimurium* in the spleen (Table 2). *S. typhimurium* was not detected in any of the tissues of the other treatment group pigs. The primary tissue colonized in the control group was the colon (71% or 5/7) and the lymph node was the second most commonly colonized tissue (43% or 3/7). The liver and spleen were both colonized in 14% (1/7) of the pigs in the control group. Although the reason that the colon was the primary tissue colonized might have been surface bacteria, the lack of colonization in the treatment group and the careful washing of all tissues before disruption should have eliminated them.

No differences were detected in the shedding duration or amounts between the treatment or control group (Tables 3 and 4). The failure to detect differences probably was due to the small sample size and the short duration of the experiment. No difference in weight gain was detected between the two groups. Initial screening of tissues failed to isolate L-form bacteria.

After clinical signs ceased, pigs in both groups continued to shed *S. typhimurium*. The odds of finding *S. typhimurium* in the tissues were significantly ($P=.009$) lower for pigs given antibiotics at subtherapeutic and therapeutic levels than for the control group. This provides evidence that the use of antibiotics in the treatment group prevented invasion of *S. typhimurium* into tissues. These results have important food safety implications.

Table 1. Percentage of Pigs Shedding on Day 25 and Colonized in at Least One Tissues by *Salmonella typhimurium*

Group	Pigs	Shedding on Day 25	Colonized (at least 1 tissue)
Treated	9	22%[a]	11%[a]
Control	7	57%[a]	86%[b]

[a]No statistical difference ($P \leq .05$) was detected between percents in a column that share the same superscript.

Table 2. Percentage of Pigs from which *Salmonella typhimurium* Was Isolated from Swab Samples of Intestinal Tissue

Group (pigs)	Jejunum	Ileum	Spiral Colon	Cecum	Colon	Ileo-Cecal
Control (8)	75[a]	75[a]	100[a]	88[a]	100[a]	88[a]
Treated (9)	11[b]	22[a]	67[a]	89[a]	89[a]	89[a]

[a]No statistical difference ($P \leq 05$) was detected between percents in a column that share the same superscript.

Table 3. Average Shedding Score (Rounded to Nearest Whole Number) for *Salmonella typhimurium* Isolated from Rectal Swabs

	Days Postinoculation									
Group	3	5	7	11	13	15	18	20	22	25
Control	1[a]	1	1	2	2	2	1	2	1	2
Treated	2[b]	1	1	2	2	2	2	2	2	3[c]

[a]1 = Isolated from direct plate.
[b]2 = Isolated from enrichment.
[c]3 = Isolated from double enrichment.

Table 4. Average Shedding Score (Rounded to Nearest Whole Number) for *Salmonella typhimurium* Isolated from Tissue Sections

Group (pigs)	Ileum	Ileo-Cecal	Cecum	Jejunum	Colon	Spiral Colon
Control (7)	2[a]	2	2	2	2	2
Treated (9)	3[b]	3	3	3	3	2

[a]2 = Isolated from enrichment.
[b]3 = Isolated from double enrichment.

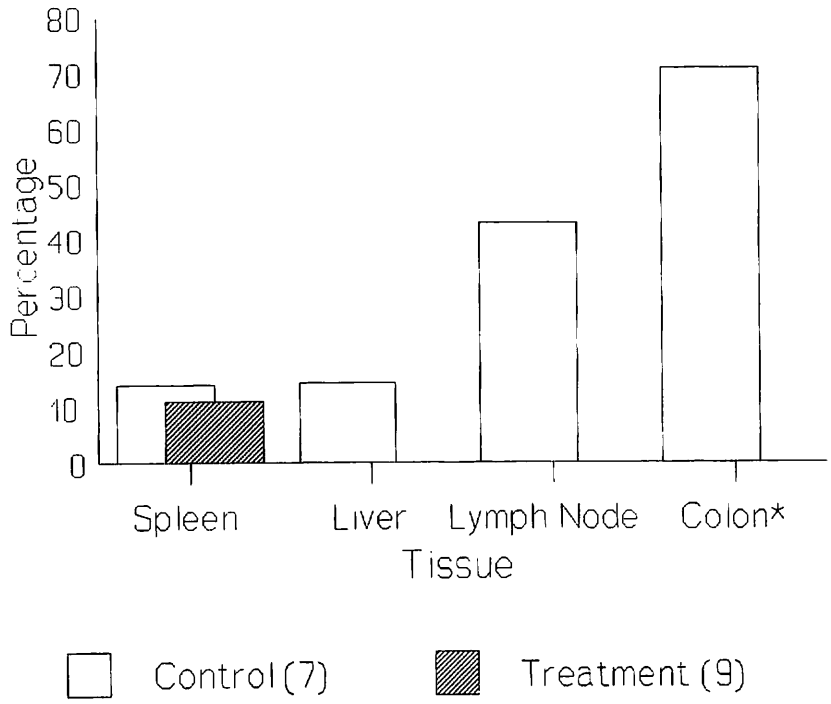

Figure 1. Percent of Pigs in Each Group with *Salmonella typhimurium* Isolated from Four Tissues. Asterisk Indicates Significant ($P \leq .05$) Difference between Control and Treatment Group