Effects of tylosin administration on C-reactive protein concentration and carriage of Salmonella enterica in pigs

Hyeun Bum Kim, DVM, PhD; Randall S. Singer, DVM, PhD; Klaudyna Borewicz, MS; Bryan A. White, PhD; Srinand Sreevatsan, DVM, PhD; Timothy J. Johnson, PhD; Luis A. Espejo, DVM, PhD; Richard E. Isaacson, PhD

**Objective**—To evaluate the effects of tylosin on C-reactive protein concentration, carriage of *Salmonella enterica*, and antimicrobial resistance genes in commercial pigs.

**Animals**—120 pigs on 2 commercial farms.

**Procedures**—A cohort of sixty 10-week-old pigs in 4 pens/farm (15 pigs/pen) was randomly selected. Equal numbers of pigs were given feed containing tylosin (40 µg/g of feed) for 0, 6, or 12 weeks. C-reactive protein concentrations were measured, microbial culture for *S enterica* in feces was performed, and antimicrobial resistance genes in feces were quantified.

**Results**—No significant associations were detected between C-reactive protein concentration or *S enterica* status and tylosin treatment. During the 12 weeks of tylosin administration, increased levels of 6 antimicrobial resistance genes did not occur.

**Conclusions and Clinical Relevance**—Treatment of pigs with tylosin did not affect C-reactive protein concentration or reduce carriage or load of *S enterica*. There was no evidence that pigs receiving tylosin had increased carriage of the 6 antimicrobial resistance genes measured.

**Impact for Human Medicine**—*S enterica* is a public health concern. Use of the antimicrobial growth promoter tylosin did not pose a public health risk by means of increased carriage of *S enterica*. (Am J Vet Res 2014;75:460–467)

---

**Antimicrobials** have been used in animal agriculture for more than 60 years and have contributed to the improvement of livestock health and productivity. Antimicrobials led to livestock at subtherapeutic doses promote growth by improving feed efficiency. The mechanism of growth promotion is unknown but probably includes alterations in the composition of the intestinal microbiome of animals.

The long duration of antimicrobial use at subtherapeutic concentrations contributes to the selection and maintenance of antimicrobial-resistant bacteria. Recently, the FDA Center for Veterinary Medicine issued Guidance for Industry documents #209 and #213, stating that AGPs should be phased out of animal agriculture in the United States. Antimicrobial growth promoters are thought to have additional beneficial effects in animals such as better health, reduced morbidity and mortality rates, reduction of subclinical infectious diseases, and reduced risk of carrying zoonotic pathogens. All of these contribute to the production of animals that are healthier and more uniform in size at the time of slaughter, which may lead to decreased processing errors in slaughter plants and reduced carcass contamination, thereby improving human health. Tylosin is one of the most commonly used AGPs in pork production. Tylosin has antimicrobial activity against most gram-positive bacteria, *Mycoplasma* spp, and a limited ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGP</td>
<td>Antimicrobial growth promoter</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
</tbody>
</table>
number of gram-negative bacteria. Although tylosin does not have direct antimicrobial activity against *Salmonella enterica*, tylosin reduces the duration of shedding in pigs experimentally challenged with *Salmonella enterica* serovar Typhimurium. In swine production, tylosin is also used therapeutically for several diseases including atrophic rhinitis and proliferative enteropathy. The FDA considers the macrolide class of antimicrobials to be critically important in human medicine.

*Salmonella enterica* is one of the most important bacterial foodborne pathogens and is highly prevalent in pig herds. Pork products are known to be a source of this pathogen. There is substantial evidence that tylosin alters the composition of the intestinal microbiome in animals. A healthy intestinal microbiome should be capable of excluding pathogens such as *Salmonella enterica*. Also, CRP is an indicator of swine health. Therefore, the purpose of the study reported here was to evaluate the effects of tylosin on CRP concentration, carriage of *Salmonella enterica*, and antimicrobial resistance genes in commercial pigs.

### Materials and Methods

**Study design**—Pigs on 2 commercial farms in southwest Minnesota were used in this study. Pigs were kept in wean-to-finish barns. These sites were selected because pigs could be housed in 1 building for the entire experimental period without relocation and would be exposed to the same microbes. The barns contained 20 pens, and each pen contained 25 pigs. Pigs were kept in the same pen during the entire sampling period without introduction of any new pigs. On each farm, a cohort of 60 pigs was randomly selected and allocated to 4 pens (15 pigs/pen). On each farm, 2 pens with tylosin-treated pigs were adjacent to each other on 1 side of the building (pens 1 and 2), and 2 pens with pigs that were not treated with tylosin (pens 3 and 4) were adjacent to each other and directly across from the pens with tylosin-treated pigs. Beginning at 10 weeks of age, pigs in pens 3 and 4 received feed containing tylosin. At 16 weeks of age, pigs in pen 1 received feed without tylosin. At 22 weeks of age, the study ended when pigs were 22 weeks of age. None of the pigs in either group received any additional therapeutic or nontherapeutic antimicrobials. This study was approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

**Sample collection**—Pigs were ear-tagged for identification, and fresh fecal samples were individually collected from the rectum of each pig. Samples were collected 5 times at 3-week intervals starting when the pigs were 10 weeks old. For measurement of serum CRP concentration, 4 pigs in each pen from 1 farm were randomly selected, and blood was collected from the same pigs 5 times at 3-week intervals starting when the pigs were 10 weeks old.

**Measurement of serum CRP concentration**—Serum CRP concentration was measured with a commercial pig CRP ELISA quantification kit. Procedures were performed according to the manufacturer’s instructions.

**Detection and quantification of *Salmonella enterica***—Salmonella enterica in feces was quantified with an MPN protocol as described. One gram of feces was serially diluted 5-fold in tetraethionate broth. One milliliter of each dilution was incubated at 41°C for 24 hours in triplicate. One hundred microliters of each dilution was then transferred to 900 µL of Rappaport-Vassiliadis R10 broth and incubated for 24 hours at 37°C. The Rappaport-Vassiliadis R10 broth was streaked on xylose-lysine tergitol agar (XLT4) plates and incubated at 37°C for 24 hours. Suspect *Salmonella enterica* colonies were confirmed by use of a test kit and PCR assay with primers specific for the gene invA (forward: AGAGTGCCTGTTTACGACCTGAAT; reverse: AGAGCAGTGCTGACTGATCGAGTAAAT). Template DNA was prepared from *Salmonella enterica* isolates with a boiling lysis procedure. The PCR mixtures contained 12.5 µL of master mix, 0.2 µM primer pairs, and 2 µL of DNA template in a 25-µL reaction. Cycling conditions started with an initial denaturation at 95°C for 2 minutes, followed by 30 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. A final 7-minute extension was performed at 72°C.

**PFGE and serotyping**—The PFGE was performed as described by use of *Salmonella enterica* isolates obtained from the pig feces. Briefly, PFGE agarose plugs containing whole *Salmonella enterica* DNA were prepared with agarose, and the plugs were digested for 18 hours with 50 U of the restriction enzyme XbaI. The plugs were then placed in a 1% agarose gel and separated by electrophoresis for 18 hours with an electrophoresis system. The electrophoresis conditions were as follows: initial switch time value of 2.2 seconds and final switch time of 63.8 seconds at a gradient of 6 V/cm, which included an angle of 120°. After electrophoresis, the gel was stained with ethidium bromide solution (50 µg/mL) for 20 minutes. The DNA from *Salmonella enterica* serovar Braenderup H9812 was used as a molecular size standard. The *Salmonella enterica* isolates obtained from the pig feces were serotyped by use of PFGE and serotyping.

**Figure 1**—Schematic representation of the design of a study of the effects of tylosin on CRP concentration and carriage of *Salmonella enterica* in pigs.
PFGE banding patterns were analyzed with analysis software. A dendrogram was generated with the Dice coefficient and unweighted pair group method by use of the arithmetic mean (UPGMA), with the analysis parameters based on 0.5% of optimization and band-matching tolerance. Representative isolates from each PFGE type were submitted to National Veterinary Services Laboratories for serotyping.

Measurement of MIC—Antimicrobial susceptibility testing of each *S. enterica* isolate against 15 antimicrobials was conducted with a broth microdilution method. The MIC breakpoints were based on Clinical and Laboratory Standards Institute recommendations. *Escherichia coli* ATCC 25922 was used for quality control purposes.

Quantification of antimicrobial resistance genes in pig feces—Total DNA was extracted from each fecal sample as described and used for detection of 6 antimicrobial resistance genes. Primers against *flo* (florphenicol), *cmy* (cephalosporin), *ermB* (macrolide), *mefA* (macrolide), *tetA* (tetracycline), and *tetM* (tetracycline) were used to amplify each specific gene. Each qPCR reaction contained 100 ng of fecal DNA and was supplemented with 10 µg of BSA. Samples were amplified by use of real-time PCR. The PCR conditions were 10 minutes at 95°C, followed by 40 cycles of 95°C for 30 seconds, 60°C for 1 minute, and 72°C for 30 seconds. Gene-specific standards at known concentrations were included in each sample plate and used to calculate the copy number of each gene in each DNA sample. Standard curves were prepared by determining mean values from all runs, and line of best fit equations were used to calculate quantities of each gene in the unknown samples.

Statistical analysis—Logistic regression with repeated measurements was used to model the probability of *S. enterica* infection. The initial model included treatment, time, and farm as explanatory variables and all possible 2-way interactions as explanatory variables. Reduction of models was performed by use of backward elimination of nonsignificant interaction terms with a value of *P* > 0.05. Main effects including treatment, time, and farm were forced to stay in the model because they were the variables of interest. Compound symmetry was used to model the working correlation structure of observations within each subject used, and it was chosen by use of the Akaike information criterion. Model assumptions were evaluated by use of residual plots. Adjustment for multiple comparisons was performed with the Tukey-Kramer procedure. Models were fitted with the mixed procedure in the statistical software.

**Results**

Serum CRP concentration—The concentration of serum CRP was measured as a biomarker of health.

![Figure 2](image-url) **Figure 2**—Mean ± SD serum CRP concentrations measured at 5 time points in pigs that did (white bars; *n* = 4) or did not (black bars; *n* = 4) receive tylosin in the feed.

![Figure 3](image-url) **Figure 3**—Numbers and percentages (denominator, 30) of pigs that did (white bars) or did not (black bars) receive tylosin in the feed and shed *S. enterica* on 2 farms. A—Farm 1. B—Farm 2.
C-reactive protein concentrations decreased over the sampling times \((P < 0.001)\), but there was no significant \((P = 0.07)\) association between the concentration of CRP and treatment with tylosin (Figure 2). Mean ± SD serum concentrations of CRP in tylosin and no-tylosin groups (ie, the groups that were fed or not fed tylosin for the entire study period) were 65.52 ± 6.93 \(\mu\)g/mL and 64.07 ± 8.47 \(\mu\)g/mL, respectively, and were within the range observed in clinically normal pigs (≤ 80 \(\mu\)g/mL).\(^{19}\) Data from the crossover groups were not graphed because there were no differences in CRP concentrations between groups that were fed or not fed tylosin for the entire study period.

Detection of pigs shedding \(S\) enterica and quantification of \(S\) enterica load—The prevalence of pigs shedding \(S\) enterica was greater in younger pigs than older pigs at both farms, regardless of treatment with tylosin (Figure 3). A logistic regression model with repeated measurements revealed that there was a significant \((P = 0.01)\) decrease in the odds of \(S\) enterica shedding over the sampling time period. The prevalence of pigs shedding \(S\) enterica was greater on farm 2 than farm 1. The odds of shedding \(S\) enterica were 0.16 (95% CI, 0.06 to 0.42) for pigs on farm 1, compared with results for farm 2 (\(P = 0.01)\). No significant \((P = 0.86)\) association was found between the prevalence of pigs shedding \(S\) enterica and tylosin treatment. The odds ratio of shedding \(S\) enterica for pigs that did not receive tylosin was 1.06 (95% CI, 0.59 to 1.90; \(P = 0.86)\), compared with results for pigs that received tylosin. The adjusted probabilities of shedding \(S\) enterica were 16.7% and 3.2% for pigs that did or did not receive tylosin, respectively.

The MPN analysis revealed that the number of \(S\) enterica in fecal samples was greater in younger pigs.
than older pigs. In general, the concentration of S. enterica decreased over time regardless of whether pigs were treated with tylosin (Figure 4). No significant association was found between the number of S. enterica and tylosin treatment (P = 0.49) or farm (P = 0.25). The geometric mean of the MPN for farm 1 was 5.48 (95% CI, 2.49 to 12.05), whereas this value was 9.33 (95% CI, 5.90 to 14.76) for farm 2. The geometric means of the MPN for S. enterica were 8.20 (95% CI, 4.51 to 14.93) and 6.23 (95% CI, 3.63 to 10.68) for pigs that did or did not receive tylosin, respectively. However, variation of the natural log-transformed S. enterica MPN was associated with time (P = 0.01).

S. enterica PFGE and serotyping—To evaluate whether treatment with tylosin affected the diversity of S. enterica serotypes, PFGE and serotyping were used to characterize each S. enterica isolate. Five PFGE types were identified from farm 1, and 2 PFGE types were identified from farm 2 (Figure 5). Seven representatives of each PFGE type were serotyped. Of these serotypes, the 2 farms shared only S. Typhimurium. In pigs from farm 1, S. Infantis was the most abundant serotype, comprising 53.85% of all S. enterica isolates, followed by S. Typhimurium at 15.38%. In pigs from farm 2, S. Infantis was the most abundant serotype, comprising 51.85% and 48.15% of the S. Typhimurium and S. Manhattan. These 2 serotypes comprised 51.85% and 48.15% of the S. enterica isolates from farm 2. Four pigs shed different S. enterica PFGE types and S. enterica serotypes at different time points, which indicated cocirculation with different types of S. enterica during the sampling periods. The same S. enterica PFGE types and serotypes were detected from the same pigs throughout the experimental period regardless of tylosin treatment.

Antimicrobial susceptibility—Fifteen S. enterica isolates that represented at least one of each of the serotypes were tested for susceptibility to 15 antimicrobials. The S. enterica isolates used in the MIC test were

<table>
<thead>
<tr>
<th>Farm</th>
<th>Serotype</th>
<th>Group</th>
<th>FOX</th>
<th>AMI</th>
<th>CHL</th>
<th>TET</th>
<th>AXO</th>
<th>AUG</th>
<th>CIP</th>
<th>GEN</th>
<th>NAL</th>
<th>TIO</th>
<th>SXN</th>
<th>KAN</th>
<th>AMP</th>
<th>STR</th>
<th>TYL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infantis</td>
<td>T</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>&lt;4</td>
<td>&lt;0.25</td>
<td>&lt;1/0.5</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.39</td>
<td>8</td>
<td>&lt;1</td>
<td>&lt;32</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Infantis</td>
<td>NT</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>&lt;4</td>
<td>&lt;0.25</td>
<td>&lt;1/0.5</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.40</td>
<td>8</td>
<td>&lt;1</td>
<td>&lt;32</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Infantis</td>
<td>T</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>&lt;4</td>
<td>&lt;0.25</td>
<td>&lt;1/0.5</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.41</td>
<td>8</td>
<td>&lt;1</td>
<td>&lt;32</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Infantis</td>
<td>NT</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>&lt;4</td>
<td>&lt;0.25</td>
<td>&lt;1/0.5</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.42</td>
<td>8</td>
<td>&lt;1</td>
<td>&lt;32</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Livingstone</td>
<td>T</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>8/4</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.43</td>
<td>&gt;84</td>
<td>&lt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Livingstone</td>
<td>NT</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>&lt;4</td>
<td>&lt;0.25</td>
<td>&lt;1/0.5</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.38</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>NT</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.38</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>T</td>
<td>2</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>8/4</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;0.12/2.38</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>NT</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>8/4</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>T</td>
<td>2</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>8/4</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>NT</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>T</td>
<td>2</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Manhattan</td>
<td>NT</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>8/4</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Manhattan</td>
<td>T</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Manhattan</td>
<td>NT</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Manhattan</td>
<td>T</td>
<td>4</td>
<td>2</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Typhimurium</td>
<td>T</td>
<td>8</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>2</td>
<td>Typhimurium</td>
<td>NT</td>
<td>8</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&lt;0.25</td>
<td>16/8</td>
<td>0.03</td>
<td>&lt;0.25</td>
<td>4</td>
<td>1</td>
<td>&lt;4/76</td>
<td>&lt;8</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>


Shaded values indicate resistance; values in boxes indicate intermediate resistance.
not susceptible to tylosin (MIC > 1,000 µg/mL). Susceptibility testing of 15 other antimicrobials revealed that all S enterica isolates were susceptible to cefoxitin, amikacin, ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid, and ceftiofur (Table 1). Salmonella Infantis isolates from farm 1 and 1 S Typhimurium isolate from farm 2 were susceptible to all 15 antimicrobials tested. The other S enterica serotypes were resistant to 2 or more antimicrobials. The other S Typhimurium isolates from farm 2 were resistant to 5 antimicrobials: ampicillin, chloramphenicol, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline (the classic ACSSuT phenotype32). The S Typhimurium isolates from farm 1 were resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline but were susceptible to the sulfas. The S Manhattan isolate was resistant to ampicillin, trimethoprim-sulfamethoxazole, and tetracycline.

Quantification of antimicrobial resistance genes in pig feces—Quantification of 6 antimicrobial resistance genes was performed to determine whether, during the 12 weeks of the study, tylosin treatment resulted in increases in the concentrations of these resistance genes or whether antimicrobial-free pigs had lower concentrations. The 6 genes (flo, ermB, tetM, cmy2A, mefA, and tetA) were selected as representatives of major classes of antimicrobials that also might have genetic linkages. There were no differences between groups regardless of treatment, including the 2 crossover groups (Figure 6). Both flo and tetA were present at higher concentrations in pigs at 10 weeks of age, and concentrations decreased markedly over time. Concentrations of ermB, tetM, and mefA remained constant through the sampling period.

Discussion

Antimicrobials have been used extensively as AGPs in agricultural animal production. One of the hypothesized benefits of AGPs is that they reduce the prevalence of infectious diseases by decreasing pathogen prevalence and loads in animals.1,33–36 The study reported here was based on the hypothesis that tylosin, a commonly used AGP in pig production, would reduce carriage of S enterica and thus reduce the potential for spread of zoonotic pathogens such as S enterica.

Serum CRP concentration has been used as an indicator of infections in humans and to assess the health status of swine.18,37–39 In pigs, serum CRP concentrations are correlated with the presence of clinical infectious diseases.19 Longitudinal analysis of CRP concentrations in pigs from farm 2 revealed that CRP concentrations decreased in pigs as their age increased. However, there was no greater association between tylosin treatment and serum CRP concentration, compared with untreated controls. Therefore, our results indicated that tylosin treatment was not associated with CRP concentrations in healthy pigs.

We also evaluated whether tylosin was associated with a decrease in the prevalence and load of the zoonotic pathogen S enterica. Pigs on both farms in this study were naturally infected with S enterica. Results indicated that the prevalence and load of S enterica decreased as pigs grew older regardless of tylosin treatment. Treatment with tylosin was not associated with any additional reduction in the number of pigs infected with S enterica or the load of S enterica in feces. Thus, in this setting, our hypothesis was not supported. Results of previous studies40–41 including experimental challenge studies,15,42–46 corroborate these findings. However, Shryock et al15 did determine that administration of tylosin reduced the duration of S enterica shedding in experimentally infected pigs.

A narrow range of S enterica serotypes was detected in the feces of pigs in the study reported here. The same S enterica PFGE types and S enterica serotypes were detected from the same pigs throughout the experimental period regardless of tylosin treatment. There was no association between S enterica diversity and tylosin treatment, indicating that tylosin treatment was not associated with selection of a certain S enterica type.

One of the concerns about usage of AGPs is that it can result in selection for antimicrobial resistance genes. Unlike studies of the antimicrobial resistance profile of specific bacteria, the present study quantitatively measured antimicrobial resistant genes by use of community DNAs in pig feces in addition to determining the antimicrobial-resistant profile of the S enterica that was detected. During the 12-week study, no significant differences in the concentrations of the 6 antimicrobial resistance genes were detected in either group. In Europe, where a ban on AGPs was instituted in 1999, data revealed marked reductions in the concentrations of antimicrobial resistance genes in Enterococcus faecium and Enterococcus faecalis, particularly against macrolides, lincosamides, and tetracyclines 5 to 6 months after the ban.47,48 However, those data were determined in animals at slaughter. In the present study, the entire microbial community in the feces was assessed for 6 antimicrobial resistance genes, rather than selected bacterial species. The present study determined antimicrobial resistance gene concentrations for a period of 12 weeks during the growth of pigs, compared with animals at slaughter.47,48 and determined antimicrobial gene load in the entire fecal microbiome rather than in a single genus (ie, Enterococi spp). Because the study period was restricted to 12 weeks, it is possible that a longer evaluation might have revealed a reduction in concentrations of antimicrobial resistance genes in some of the tylosin-treated pigs. However, the time period was selected because it represented the time period when pigs in typical production units would be approaching market weight. Both of these differences in study design could explain the differences observed between the present study and previous studies. However, although Enterococi spp are important pathogens in humans and selection of resistant clones can present potential treatment difficulties, evaluating resistance genes in a single genus does not provide a broad picture of resistance gene carriage or the effects of exposure to antimicrobials.

If nontreated pigs retain a high gene copy number of resistance genes because of their carriage in other intestinal bacteria, the potential remains high that gene transfer to pathogenic bacteria will occur when bacteria are exposed to antimicrobials.
Results of this study indicated that treatment of pigs with tylosin did not affect CRP concentrations or reduce carriage or load of S enterica. Under the constraints of this field-based study, tylosin did not increase the carriage of 6 antimicrobial resistance genes. These results were obtained from healthy pigs on 2 farms in Minnesota. Whether the same results would have been obtained from unhealthy pigs or in other geographic locations remains unknown.

References


