INFLUENCE OF BAMBERMYCINS ON SALMONELLA INFECTION AND ANTIBIOTIC RESISTANCE IN CALVES

Jean Dealy and M. W. Moeller

Litton Bionetics, Inc., Kensington, MD 20795 and Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876

SUMMARY

Fourteen 8-week-old calves infected with S. typhimurium were fed control and 10 mg/head/day bambermycins in an 8-week study to determine the effect on quantity, prevalence, shedding and susceptibility of Salmonella. Special precautions were taken to eliminate cross-contamination between infected and uninfected animals on both treatments.

Salmonella counts of the homogenized fecal samples were monitored to study the parameters before and after inoculation. Five colonies from each fecal specimen suspected of being Salmonella were isolated, serologically identified and tested for susceptibility to 10 antibiotics.

The use of bambermycins supplemented feed reduced the duration and prevalence of Salmonella shedding in calves. Bambermycins fed calves showed a significant decrease in the number of Salmonella shed during the first 7 days, and throughout the trial the quantity of Salmonella shed was less. Furthermore, feeding bambermycins diets significantly reduced the number of Salmonella resistant to streptomycin, ampicillin, and oxytetracycline.

(Key Words: Bambermycins, Feed Additive, Salmonella, Resistance, Antibiotic, Cattle.)

INTRODUCTION

The occurrence of multiple resistance of the transferrable type in strains of S. typhimurium isolated from pigs and humans was reported by Anderson (1965). Moreover, resistance was most common among strains of S. typhimu-

1 We gratefully acknowledge the technical assistance of Eva Barrion. Thanks are also due to Charles Riggs and his staff for the statistical analyses.

2 Also referred to as Flavomycin®; registered TM-Hoechst AG.
content were both negative at the beginning of the experiment. The medicated test feed was the same cattle developer supplemented with 44.0 mg bambermycins per kilogram (Grant et al., 1974). Quantitative antibiotic assays run at the beginning of the experiment confirmed this bambermycins concentration. The calves were fed twice daily. Nonmedicated feed was fed except that the medicated calves only received 227 g of bambermycins supplemented feed (10 mg bambermycins) each morning. Hay and water were given ad libitum.

Salmonella typhimurium (S. typhimurium calf strain DVR-76), kindly supplied by the Division of Veterinary Medical Research, Food and Drug Administration, Beltsville, MD, was used as the infecting organism. This organism belongs in serogroup B, and the antibiogram for this strain is given below:

\[
\begin{array}{ccccccccc}
R & S & S & S & S & R & S & R & R & S & S & F \\
\end{array}
\]

A-ampicillin, C-chloramphenicol, N-nitrofurantoin, G-gentamycin, S-streptomycin, Ne-neomycin, T-terramycin (oxytetracycline), SSS-sulfonamides (triple sulfa), Cl-colistin (polymyxin), P-bambermycins (Flavomycin). Sensitivity (S) and Resistance (R) are based on Bauer (1966) method. DVR-76 carried plasmid resistance to ampicillin, streptomycin, oxytetracycline, and triple sulfa (L. D. Robbins, unpublished data).

The calves were randomly allotted by weight into four treatment groups. Seven calves were assigned to the Salmonella infected Treatment Groups A (nonmedicated feed) and B (bambermycins medicated feed). Three calves were assigned to the two identically treated uninfected environmental control groups included in order to monitor for cross-contamination. Management practices were the same for all groups. Precautions were taken to restrict the movements of personnel to avoid the possibility of cross-contamination.

The S. typhimurium strain used for infecting the treatment groups was previously titrated to ensure that a sufficient number of organisms was introduced to facilitate colonization. The calves in Treatment Group B were fed the medicated ration containing bambermycins for 5 days prior to inoculation. All 14 animals in Groups A and B were inoculated at approximately 8 weeks of age. The animals were fasted overnight before infection with S. typhimurium by gavage. A plate count of the inoculum using Brilliant Green Agar was made following infection. Each animal received \(3.0 \times 10^{12}\) organisms.

Fecal samples were taken from all calves on days 2, 4, 7, 10, 14, 21, 28, 35, 42, 49 and 56 postinfection. Specimens were processed as soon after collection as possible. \(Salmonella\) typhimurium counts were made on homogenized fecal samples by methods previously described (Dealy and Moeller, 1976).

Five colonies from each fecal specimen suspected of being \(Salmonella\) were biochemically and serologically identified by the methods of Falkow (1958) and Edwards and Ewing (1962). These isolates were tested for susceptibility to antibacterials according to methods previously described using the same selection of antibiotic discs (Dealy and Moeller, 1976).

Fifty-six days postinoculation, all calves were killed and necropsied. Liver, spleen, ileocaecal lymph node and cecum were aseptically collected in that sequence. One gram of each tissue was minced and cultured in 9 ml Tetra-thionate Brilliant Green Broth for 48 hours. Broth cultures were then transferred to Brilliant Green Agar for \(Salmonella\) isolation and identification.

### TABLE 1. GEOMETRIC MEAN NUMBER\(^a\) OF \(SALMONELLA\) TYPHIMURIUM RECOVERED FROM CALVES

<table>
<thead>
<tr>
<th>Days post-infection</th>
<th>Non-medicated</th>
<th>Bambermycins</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(5.75 \times 10^6)</td>
<td>(5.82 \times 10^6)**</td>
</tr>
<tr>
<td>4</td>
<td>(8.27 \times 10^4)</td>
<td>(8.86 \times 10^4)**</td>
</tr>
<tr>
<td>7</td>
<td>(2.82 \times 10^4)</td>
<td>(3.03 \times 10^4)†</td>
</tr>
<tr>
<td>10</td>
<td>(9.61 \times 10^4)</td>
<td>(2.53 \times 10^4)</td>
</tr>
<tr>
<td>14</td>
<td>(1.03 \times 10^4)</td>
<td>(6.09 \times 10^2)</td>
</tr>
<tr>
<td>21</td>
<td>42</td>
<td>7</td>
</tr>
<tr>
<td>28</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>35</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>42</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>49</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>56</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Geometric mean = \(1.0 + \text{antilog} [\text{average log (CFU/g} + 1.0)]\).

†\(P<.08\).

*\(P<.02\).

**\(P<.01\).
RESULTS

The geometric mean of the number of organisms recovered per gram of wet feces at each sampling time for each of the two infected groups is shown in table 1. At 2 days postinfection, *Salmonella* was recovered in the feces of all infected calves and recoveries were made from all infected animals at least four times during the trial. All environmental control calves were negative of *Salmonella* throughout the trial. The mean number of *Salmonella* recovered per calf on days 2 and 4 was less (P<.01 and <.02) for the bambermycins-treated group than for the control group. At 7 days postinfection, the difference between the two groups was also large (P<.08).

Figure 1 depicts the percentage of calves shedding *Salmonella* for sampling periods 2 through 42. Generally, the number of animals shedding *Salmonella* was lower in the medicated group, the exceptions being days 2 through 10. The nonmedicated control animals were 100% positive for 14 days postinfection while the percentage of calves shedding *Salmonella* in the bambermycins-medicated group dropped to 70% by day 14. On days 14 through 42, the differences between the percentage of positive calves in the nonmedicated and the bambermycins-medicated groups were approximately 30%. By 42 days, shedding had ceased in the bambermycins-medicated calves while calves in the nonmedicated group continued to shed *Salmonella*. On days 49 and 56, all animals were negative for *Salmonella*.

Prevalence of *Salmonella* between the nonmedicated and bambermycins-medicated calves was further analyzed by comparing the proportion of animals having positive counts (i.e., greater than zero) by group for all time periods. The results are shown in table 2. For the 2 through 42-day period there was a total of 63 possible positive samples for each group. The observed number of positive samples for the nonmedicated and bambermycins-medicated groups were 48 and 39, respectively. This analysis illustrates that *Salmonella* was more prevalent (P<.02) in the nonmedicated group for the 2 through 42-day period.

Five clones from each fecal sample which was positive for *S. typhimurium* were tested for antibacterial susceptibility. Since the infecting organism remained sensitive throughout the trial to chloramphenicol, nitrofurantoin, gentamycin, neomycin and colistin these antibacterials are not included in the data. Differences were not found in the resistance patterns of *Salmonella* to triple sulfa, so it was excluded from statistical analyses.

For three antibiotics, the mean percent of positive animals carrying an antibiotic-sensitive strain of *Salmonella* was calculated for each group from days 2 through 21. The results are given in table 3. Calves fed bambermycins had greater number of *Salmonella* clones recovered from feces that were sensitive to streptomycin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmedicated</td>
<td>48</td>
<td>15</td>
<td>63</td>
</tr>
<tr>
<td>Bambermycins—medicated</td>
<td>39</td>
<td>24</td>
<td>63</td>
</tr>
</tbody>
</table>

**P<.02.
BAMBERMYCINS ON SALMONELLA INFECTION AND ANTIBIOTIC RESISTANCE

TABLE 3. PERCENT OF POSITIVE CALVES WITH SENSITIVE SALMONELLA CLONES AVERAGED OVER DAYS 2 THROUGH 21

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Non-medicated</th>
<th>Bambermycins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>5.2</td>
<td>19.1†</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>3.8</td>
<td>17.8**</td>
</tr>
<tr>
<td>Terramycin</td>
<td>18.2</td>
<td>60.0*</td>
</tr>
</tbody>
</table>

†P<.09.
*P<.05.
**P<.01.

The antibiogram data were further analyzed to determine if the percentage of sensitive Salmonella clones was the same for each group and each antibiotic. These mean percentages are shown in table 4. For streptomycin there was a difference (P<.01) between the bambermycins fed calves and the nonmedicated controls. Differences found with ampicillin (P<.08) and oxytetracycline (P<.01) were also greater.

At the conclusion of the experiment, tissues from each animal were aseptically excised and cultured to ascertain if subtherapeutic levels of bambermycins in feed increased the carrier state of Salmonella in calves. Livers, spleens, ileocaecal lymph nodes and caecal tissues from all calves were negative for Salmonella.

TABLE 4. PERCENT OF SENSITIVE SALMONELLA FROM POSITIVE FECAL SAMPLES AVERAGED OVER DAYS 2 THROUGH 21

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Non-medicated</th>
<th>Bambermycins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>1.1</td>
<td>3.6*</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>.8</td>
<td>3.5**</td>
</tr>
<tr>
<td>Terramycin</td>
<td>5.1</td>
<td>21.6†</td>
</tr>
</tbody>
</table>

†P<.01.
*P<.05.
**P<.01.

Discussion

This investigation illustrated that feeding bambermycins at 10 mg/head/day to 8-week-old calves experimentally infected with S. typhimurium decreased the number of organisms shed during the first 7 days after infection (P<.08) and decreased the total duration of time that Salmonella was shed. Generally, the quantity of shedding was less for the bambermycins-medicated group with the differences being the greatest on days 2 and 4 (P<.01 and <.02). Additionally, the prevalence of Salmonella in these calves was also reduced (P<.02).

Similar studies using chlortetracycline illustrated that the pattern and the number of S. typhimurium recovered in feces of calves were not altered significantly by continuous daily chlortetracycline feeding (Royal et al., 1970). Finlayson and Barnum (1973) reported similar findings in swine. However, results of investigations reported elsewhere (Dealy and Moeller, 1976) showed that bambermycins feeding greatly reduced both the quantity and prevalence of S. typhimurium in experimentally infected swine.

Investigations where low levels of bambermycins were added to swine feed further demonstrated that bambermycins reduced antibiotic resistance in E. coli (Federic and Sokol, 1973) and S. typhimurium (Dealy and Moeller, 1976). The results reported here also illustrate that the addition of bambermycins to the diet of calves decreased the number of organisms resistant to streptomycin, ampicillin and oxytetracycline. Since resistance to these antibiotics is R-factor-mediated, the data suggest that bambermycins decreased antibiotic resistance by elimination of R-factors. While the question of multiple resistance was not specifically addressed, the data further indicate that resistance was lost with decreasing frequency to: 1) ampicillin + oxytetracycline; 2) streptomycin + oxytetracycline; and 3) ampicillin + oxytetracycline + streptomycin.

LITERATURE CITED

Dealy, J. and M. W. Moeller. 1976. Influence of bambermycins on Salmonella infection and anti-