Subtherapeutic Tetracycline Effects on Recovery Patterns of Calves After Salmonella typhimurium Challenge

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ABSTRACT

Holstein calves were maintained on a subtherapeutic dose of chlortetracycline to determine if an oxytetracycline therapy, given after a Salmonella typhimurium challenge, would be compromised by the previous subtherapy. Two of the four groups of seven calves were maintained on a subtherapeutic amount of chlortetracycline. All calves then were challenged with Salmonella typhimurium, and with the onset of clinical symptoms one group with and one group without subtherapy were given a therapeutic dose of oxytetracycline. The two groups receiving a therapeutic dosage of oxytetracycline had the most quickly declining body temperatures and the highest average body weights postchallenge. Two calves died in the group receiving no antibiotic treatments, and one calf died in the group receiving only the subtherapeutic treatment. There were no differences in postchallenge body temperatures or body weight changes between subtherapeutic and nonsubtherapeutic groups of calves. The conclusion was that the subtherapeutic dosing of chlortetracycline did not affect the therapeutic treatment effects of oxytetracycline after a Salmonella typhimurium challenge.

INTRODUCTION

The feeding of subtherapeutic amounts of antibiotics to domesticated animals has been practiced since the early 1950's, and economic benefits of an increased rate of gain resulting in an improved feed efficiency by the control of subclinical disease infections are documented (3). However, concern has been expressed regarding the emergence of strains of bacteria resistant to single and multiple antibiotics from the use of antibiotics as feed additives for disease control and growth promotion (2, 7). Smith (8) has given some evidence that humans heavily exposed to the more commonly used antibiotics do have a higher incidence of the antibiotic resistant bacteria as compared to an unexposed population. To date there is no evidence directly linking the resistant bacteria pool in man to antibiotic-fed animals or products from such animals (3). Manten (4) has noted that widespread use of antibiotics in medical and veterinary treatments, as well as their use in animal feeds, has not evolved into a serious medical problem of drug resistance over the last decade. A possible reason for continued effectiveness of these antibiotics is that organisms that are more competent to receive the resistance factors are, in fact, less virulent (5). Fagerberg and Quarles (1) have reviewed the literature on development of antibiotic resistance and potential compromises of feeding subtherapeutic antibiotics to food-producing animals.

The objective of this study was to determine whether calves previously maintained on an antibiotic subtherapy would respond to a therapeutic treatment of antibiotics after a disease challenge.

METHODS AND MATERIALS

Twenty-eight Holstein calves of unknown age, weighing approximately 70 kg from local dairy farms were divided into four groups of seven and housed in four separate 6 x 6 m isolation rooms at the CSU Contagious Disease Laboratory. The equipment, animals, and caretaker's clothing remained within each isolation room throughout the entire experi-
TABLE 1. Experimental design.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of calves</th>
<th>Subtherapeutic treatment</th>
<th>S. typhimurium challenge dose</th>
<th>Therapeutic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>7</td>
<td></td>
<td>2 ml</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>7</td>
<td></td>
<td>2 ml 11.1 mg OTC</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>7</td>
<td>70 mg CTC</td>
<td>2 ml</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>7</td>
<td>70 mg CTC</td>
<td>2 ml 11.1 mg OTC</td>
<td></td>
</tr>
</tbody>
</table>

1 Chlorotetracycline (CTC) mixed in the milk replacer (mg/calf per day). The composition of the milk replacer was: 21.0% crude protein, 10.0% fat, and 1.5% crude fiber.
2 Oral dose of Salmonella typhimurium, strain D/S #74 (5.2 × 10^10 organisms/ml/calf).
3 Oxytetracycline (OTC) injected i.m. (mg/kg BW per day).

Each of the four groups was assigned to a combination of subtherapy and therapy treatments, which resulted in a 2 × 2 factorial design (Table 1). Two groups were fed subtherapeutic quantities (70 mg/calf per day) of chlorotetracycline (CTC) added to the milk replacer throughout the entire 38-day experiment. The remaining two groups did not receive a CTC subtherapy. After 21-day adjustment, all calves were given orally a 2-ml dose containing 5.2 × 10^10 viable S. typhimurium (strain D/S #74) organisms per milliliter. Within 48 h of inoculation, all groups of calves demonstrated clinical symptoms, and at that time two groups of calves (one group with and one group without CTC treatment) were treated with a therapeutic intramuscular (i.m.) dose (11.1 mg/kg BW (body weight)) of oxytetracycline (OTC) daily for 3 days.

Fecal samples were collected from the rectum of each calf with a rubber glove for Salmonella counts, and degree of scouring was noted at 46 to 47 h postchallenge (Table 2). Approximately 1 g of feces from each calf was collected in a sterile manner, homogenized in 5 ml of tetrathionate broth, maintained in an ice water bath, and sent immediately to the laboratory. Fecal analysis began immediately upon arrival at the laboratory and proceeded in the order in which the samples were collected. Each sample was diluted in a 10 serial 10-fold dilution, and .1 ml of each dilution was plated on

TABLE 2. Body temperatures, scour scores, and presumptive salmonella counts noted 48 h postchallenge.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Treatment</th>
<th>Mean body temperature (°C) (SD)</th>
<th>Mean scour score (Units) (SD)</th>
<th>Mean presumptive salmonella counts (Log^a) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Subtherapy</td>
<td>41.3 (.34)</td>
<td>4.14a (.90)</td>
<td>4.38 (1.71)</td>
</tr>
<tr>
<td>B</td>
<td>Therapy</td>
<td>41.6 (.18)</td>
<td>4.00ab (1.15)</td>
<td>4.86 (1.48)</td>
</tr>
<tr>
<td>C</td>
<td>CTC</td>
<td>41.6 (.25)</td>
<td>2.71b (1.11)</td>
<td>5.02 (1.36)</td>
</tr>
<tr>
<td>D</td>
<td>CTC</td>
<td>OTC</td>
<td>41.3 (.19)</td>
<td>2.71b (.75)</td>
</tr>
</tbody>
</table>

a,b Means with common letters within a column are not significantly different (P<.05).
1 Scour scores: 2 = soft, some form; 3 = mush, no form; 4 = watery.
2 Salmonella counts: log/g of feces.

duplicate Brilliant Green Sulfa Agar plates and incubated at 37°C for 24 h. The typical pink colonies (presumptive Salmonella) were counted on the agar plates, and Salmonella counts per gram of feces were calculated for each fecal sample (Table 2).

Body temperatures were recorded between 1500 and 1700 h, once prior to challenge and daily for 14 days postchallenge. Body weight measurements were taken at days 0, 21, 28, 35, and 38 of the experimental period. Samples of the milk replacer and calf starter were analyzed microbiologically by Diamond-Shamrock Chemical Co. for CTC content. Data were analyzed statistically by two-way analysis of variance.

RESULTS AND DISCUSSION

Results of the laboratory analysis showed that the calf starter meal did not contain CTC. However, a CTC-like antibiotic residue was in the control milk replacer at approximately 10% of the targeted CTC subtherapeutic dose. The specificity of this contaminating antibiotic could not be determined beyond identification of a tetracycline type antibiotic. The magnitude of this residue in the control milk replacer should be considered in interpreting the performance of the control (no subtherapy) group of calves.

Within 43 h postchallenge, all groups of calves had an average body temperature of 41°C or greater and, except for one calf, had scours of varying severity. Scour scores were highly variable but showed evidence of less scouring severity at this 48-h sampling point in groups previously having received the 70 mg CTC subtherapy. There were no significant differences between treatments for the presumptive Salmonella counts from the fecal samples (Table 2). Following the S. typhimurium challenge, only a few calves refused milk replacer. However, all calves refused some starter meal, a response that was markedly greater and of a longer duration by the groups not receiving OTC therapy (Table 3). Two calves died on day 6 postchallenge in the group receiving CTC but no OTC. Bacteriological diagnostic laboratory reports identified a Salmonella species isolated from the rectal lining, kidney, liver, and lymph nodes of all three calves.

All group body temperatures had returned to prechallenge body temperatures by day 7 or 8 postchallenge (Figure 1). Calves in the two groups receiving therapeutic doses of OTC had significantly (P<.01) lower average body temperature from day 3 to day 7 postchallenge (Figure 1). In contrast, the subtherapeutic CTC did not affect return of body temperature to normal.

All calves lost weight until day 7 post-
challenge (Table 4), with the exception of one group, corresponding to the return of body temperatures to prechallenge temperatures. The two groups receiving an OTC treatment had greater average body weights at days 14 and 17 postchallenge than those survivors in the no OTC groups. The subtherapeutic treatments did not affect group body weight averages. The group receiving no CTC subtherapy but an OTC therapy had the greatest \( P < .01 \) average daily gain between days 7 and 14 postchallenge (Table 4). Part of this large weight gain during the 2nd wk postchallenge was probably a reflection of a greater weight loss during the previous week due to dehydration in those calves not receiving CTC subtherapy. Calves in the group receiving only OTC therapy lost an average of 40% more body weight in the 1st wk (Table 4) postchallenge than the average body weight losses of the groups receiving a CTC subtherapy. The calves in the group receiving CTC plus OTC had a second rise in body temperature (Figure 1) at day 8 postchallenge, possibly from mild reinfection through contact with contaminated straw bedding. Chlortetracycline is a broad spectrum antibiotic that, when administered orally, could suppress the normal population of enteric bacteria of calves. Therefore, surviving pathogens in the gut or the environment may have caused the reinfection episode because of reduced normal bacterial population (6). This second rise in body temperature, although high for this group, was not significantly higher than that in other groups. This increase in body temperature lasted for approximately 4 days. No deaths occurred, and all calves in the group continued on the experiment without further incident.

**CONCLUSIONS**

The calves were affected seriously by the *S. typhimurium* challenge as demonstrated by a rise in body temperature, refused feed, scouring, and three deaths. The groups of calves receiving the therapeutic doses of oxytetracycline responded positively to this therapeutic treatment, regardless of the subtherapeutic treatment, as demonstrated by the lowered body temperatures throughout the day 3 to day 7 postchallenge. Those calves receiving a subtherapeutic dose of chlortetracycline throughout the entire experimental period were not affected adversely by the subtherapeutic treatment in their recovery from the disease challenge. Therefore, subtherapeutic dosing of CTC did not hinder therapeutic efficacy of OTC following a disease challenge in calves.

**REFERENCES**