Residues in Young Veal Calves After Consumption of Milk Containing Penicillin

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ABSTRACT
Residues in urine, kidney, and muscle following consumption of milk containing procaine penicillin G were determined in 4-to 8-day-old Holstein bull calves. Calves were fed milk diluted 1:1 with water. Nine control calves received diluted milk only while 8 calves received diluted milk containing 6,600 IU penicillin/kg (low group), and 10 calves received 13,200 IU/kg of milk consumed (high group). Urine samples were collected over 11 h after each morning feeding for 3 days. Calves were slaughtered on day 4 of trial and kidney, muscle, and urine samples obtained. Urine was tested for residues with the Live Animal Swab Test and tissues by the Swab Test on Premises. Both tests are microbiological assays used for detecting growth inhibition of Bacillus subtilis. No residues were detected in urine of control calves. Residues in urine were detected in 6 calves in the low group and 7 calves in the high group after feeding. At slaughter, residues were in 3 of 5 urine samples from calves in the low group and 8 of 10 calves in the high group. However, no residues were detected in kidney or muscle of control or treated calves.

INTRODUCTION
Treatment of mastitis and other bacterial infections with antibiotics often results in waste milk containing antibiotic residues. Disposal of this product on dairy farms usually is by feeding to young calves in either a fresh or fermented form (8). An unknown in the practice of feeding waste milk is the potential for antibiotic residues in calves slaughtered for meat (8).

Penicillin G frequently is used to treat bacterial infections in dairy cows, and its use results in detectable residues in milk (2, 10, 12, 13). Gastric acids can destroy penicillin G orally consumed (1, 6), but a portion can be absorbed and result in residues in tissues. Penicillin residues were in pigs (9) and chickens after oral consumption (4). However, little information is available on residues following oral consumption by young veal calves.

The objective of this study was to determine residues in urine and meat of young veal calves following consumption of procaine penicillin G. Screening for residues was with new tests promoted for use “on the farm” and at slaughterhouses.

MATERIALS AND METHODS
Fifteen Holstein bull calves, ranging in weight from 34 to 55 kg, were purchased from a cattle dealer on two occasions. Each time calves were assigned to three groups and balanced for body weight. One to 4 days prior to the start of each experimental period calves received either whole milk at 8% of body weight or a commercial electrolyte solution (Nordon Laboratories, Lincoln, NE) if scours were observed.

A treatment regimen was chosen to reflect management of young veal calves on dairy farms prior to slaughter. Whole milk, diluted 1:1 with H₂O, was fed twice daily at 0700 and 1600 h to 8% of body weight for 3 days. Diluted milk containing 0 (control, n=9), 6,600 IU procaine penicillin G/kg (Sigma Chemical Co., St. Louis, MO) (low, n=8), or 13,200 IU/kg (high, n=10) milk was fed to 4% of body weight at each feeding. Thus, calves in the control group received no penicillin, calves in the low group received 23,760 IU
daily, and calves in the high group received 47,520 IU procaine penicillin G daily. These amounts were chosen to simulate approximately 25 or 50% recovery of antibiotic in milk following intramammary infusion of 100,000 IU procaine penicillin G, commonly used in treatment of mastitis.

Urine samples were collected over 11 h after morning feeding during the 3-day experimental period. Plastic bottles (250 ml) were modified and suspended under each calf. A 5-ml sample collected after each urination was stored at 4°C for 24 h until analyzed for residues. Time of urination relative to morning feeding was recorded.

All calves were slaughtered at 0800 h on day 4 of each trial. Samples of urine from the bladder and pieces of kidney and muscle were collected and transported to the laboratory on ice.

Urine samples were tested for residues by the Live Animal Swab Test (LAST) (16), advocated for use on commercial dairy farms as a screening test for detecting antibiotic residues in urine of calves and cull dairy cows. Sterile cotton swabs were immersed in urine and placed on agar plates containing Antibiotic Medium No. 5 (Difco Laboratories, Detroit, MI), seeded with Bacillus subtilis (ATCC 66633) spores. A 5-µg neomycin disc (BBL, Cockeysville, MD) was included on each plate as a positive control. Plates were incubated 16 to 20 h at 29°C. After incubation, zones of inhibition around the neomycin disc and each swab were measured. The presence of any zone around swabs was considered positive for residues if the zone of inhibition around the neomycin disc was ≥ 16 mm.

Kidney and muscle samples were tested for residues by the Swab Test on Premises (STOP) (7). This test is similar to LAST, but a lower concentration of B. subtilis spores is used. The presence of any zone around swabs was considered positive for residues if the zone of inhibition around the neomycin disc was ≥ 21 mm. The STOP is promoted for use by meat inspectors to detect residues in animal carcasses at slaughter.

RESULTS AND DISCUSSION

Liquid intake, penicillin consumption, and body weight data for each group are in Table 1. Severe scours and dehydration resulted in removal of one calf from the control group and two from the low group. Average body weight of calves ranged from 44.8 kg to 45.5 kg. Total consumption of diluted milk was the same for all groups.

Observations on the number of positive urine samples from calves over the 3-day collection period are in Table 2. Residues were not in urine from calves in the control group. The LAST correctly screened all urine samples thought to be free of residues as negative. No residues were detected in urine samples from two calves in the low group, and one calf had residues on only one of three collection days. Observations were similar for calves in the high group (Table 2). Three of 10 calves in this group had no residues in urine whereas only 2 calves had residues on each of the 3 days.

Residues in urine relative to time after feeding are in Table 3. Data were grouped into 3-h blocks relative to morning feeding. Many calves urinated three to four times over the

### Table 1. Body weight and daily intake of liquid and penicillin.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>Body weight (kg)</th>
<th>Liquid consumed 1</th>
<th>Penicillin consumed (IU) 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>45.5 ± 8.5</td>
<td>3.6 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>44.8 ± 5.8</td>
<td>3.6 ± 0.5</td>
<td>23,760</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>45.1 ± 5.9</td>
<td>3.6 ± 0.5</td>
<td>47,520</td>
</tr>
</tbody>
</table>

1 1:1 mixture of whole milk and H₂O.
2 Procaine penicillin G.

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TABLE 2. Number of days that residues occurred in urine of calves fed penicillin over a 3-day period.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

11-h collection whereas others did so only one or two times. Number of calves urinating within a given period ranged from a minimum of 1 on day 3 in the high group to a maximum of 8 on day 3 in the control group. Residues were not consistently in urine of calves receiving penicillin. The number of positive urine samples within any period ranged from zero to five for calves in low and high groups. A practical recommendation on the best time to sample young veal calves for residues cannot be made from these data.

Residues in urine, kidney, and muscle of calves at slaughter after being fed 0, 23,760, or 47,520 IU procaine penicillin G daily are in Table 4. Urine samples from 4 control calves and 3 low group calves were not collected. As observed in our daily collection periods, urine from calves on the control diet was negative for residues. Furthermore, no residues were detected in kidney or muscle of control calves by STOP.

TABLE 3. Residues in urine following consumption of milk containing penicillin.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>Day</th>
<th>&lt;3 h</th>
<th>3–6 h</th>
<th>6–9 h</th>
<th>&gt;9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>1</td>
<td>0/7²</td>
<td>0/3</td>
<td>0/6</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0/5</td>
<td>0/7</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0/8</td>
<td>0/5</td>
<td>0/4</td>
<td>0/1</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>1</td>
<td>0/6</td>
<td>2/5</td>
<td>3/6</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2/5</td>
<td>2/5</td>
<td>5/6</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1/5</td>
<td>0/2</td>
<td>1/3</td>
<td>3/5</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>1</td>
<td>1/7</td>
<td>0/4</td>
<td>2/3</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5/7</td>
<td>1/4</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0/5</td>
<td>0/1</td>
<td>1/3</td>
<td>2/4</td>
</tr>
</tbody>
</table>

¹Hours after a.m. feeding.
²Calves received p.m. feeding during this period.
³No. positive/no. urinating.

Three of 5 and 8 of 10 urine samples obtained from the bladder were positive for residues in low and high groups. Thus, frequency of residues in urine was higher at slaughter than at any time during the feeding period. This confirms observations during daily urine collections that most, but not all, calves that received procaine penicillin G had residues in their urine. However, all calves positive for residues in urine were negative for residues in kidney and muscle.

Thus, daily consumption of 23,760 or 47,520 IU procaine penicillin G in a mixture of milk and water for 3 days did not result in residues in meat as assayed by STOP. We caution against extrapolating these results to other drugs or situations where young veal calves are consuming antibiotics in milk. Palmer et al. (15), detected oxytetracycline, amoxicillin, and ampicillin in serum of 5- to 10-day-old calves after oral consumption at 9, 7, and 7 mg/kg body weight, respectively. In calves consuming amoxicillin at 7 mg/kg body weight, residues in excess of those in serum were in bile, kidney, liver, and urine, while amounts less than those in serum were in muscle (14). Furthermore, the medium in which antibiotics are suspended can result in marked differences in antibiotic absorption and subsequent bioavailability. Administration of antibiotics in milk replacer markedly reduced and delayed peak serum concentrations compared with administration in water or a glucose-glycine-electrolyte solution (3, 15). Thus, differences between our study and others...
TABLE 4. Frequency of residues in urine, kidney, and muscle of calves at slaughter.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Sample (No. positive)</th>
<th>Urine</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Only five urine samples obtained.

may be due to lower intake of antibiotics (.53 or 1.05 mg/kg body weight compared with ≥ 7 mg/kg body weight), to the type of antibiotic used, to the medium that penicillin was suspended in, or to different methods of residue detection.

No residues were detected in urine from control calves by LAST, suggesting that the frequency of false positives would be minimal. Similarly, over 61% of urine samples from calves fed penicillin in milk were negative for residues. Thus, no clear relationship between presence of residues in urine and feeding of milk containing penicillin was observed. Furthermore, residues in urine at slaughter were not associated with residues in meat by STOP, supporting observations in cull dairy cows (5, 12).

Incorporation of “on farm” tests into management of dairy farms will be essential because of increased sensitivity and frequency of residue testing at slaughter. Additional research, similar to that reported here, will be required to determine the relationship between residues in urine and residues in other tissues, especially kidney and muscle. More importantly, studies should be evaluated jointly by dairy scientists, pharmacologists, veterinarians, and regulatory officials to provide the most accurate and reliable recommendations for the benefit of both consumer and dairy farmer.

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