Antibiotic residues in milk samples obtained from cows after treatment for papillomatous digital dermatitis

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Objective—To determine whether there would be detectable antibiotic residues in milk obtained from dairy cattle with papillomatous digital dermatitis (PDD) after topical treatment with oxytetracycline.

Design—Randomized controlled clinical trial.

Animals—28 lactating Holstein cows with PDD.

Procedure—Cows were assigned to 2 treatment groups. Treatment 1 (n = 16) consisted of spraying of PDD lesions with 15 ml of a solution containing 100 mg of oxytetracycline/ml; lesions were sprayed twice daily for 7 days, using a garden sprayer. Treatment 2 (n = 12) consisted of a one-time application of a bandage that consisted of cotton soaked with 20 ml of a solution containing 100 mg of oxytetracycline/ml. Milk samples were obtained before and after treatment and assayed for tetracycline content by use of high-performance liquid chromatography and a commercially available tetracycline screening test.

Results—None of the cows in either treatment group had violative residues of oxytetracycline in milk samples.

Conclusions and Clinical Relevance—Producers treating lactating cows that have PDD, via topical application of oxytetracycline solution at the concentrations reported in this study, have a low risk of causing violative antibiotic residues in milk. (J Am Vet Med Assoc 1999;215:833–836)

Digital dermatitis or papillomatous digital dermatitis (PDD) was first reported in Italy in 1974 and in New York in the late 1970s. The disease has spread throughout much of North America, especially the United States and Mexico. Although the exact cause of this disease is unknown, spirochete-like organisms have been observed on specimens examined by use of silver stain, and other organisms are believed to be involved with the disease. Diagnostic evidence does not support viral involvement. Lesions begin as an eroded area, usually between the bulbs of the heels, which, if untreated, progresses with tissue proliferation and outgrowths of dermal tissue that grossly resemble hair, thus, the common name hairy heel wart.

Because of the location of these painful lesions, cattle move reluctantly and shift their weight off the heel and to the toe of the affected foot. Feed intake is reduced, and daily milk production decreases. Signs of estrus are reduced, and number of days from parturition to conception increases. There are costs associated with treatment as well as for discarded milk when treatments pose antibiotic residue problems.

Treatment of individual cows in a dairy barn hospital area is labor intensive, costly, and probably not feasible when outbreaks involve a large number of cattle in a herd. Parenteral administration of antibiotics has been used effectively in herds in California, but other authors have not had similar results. Topical application of oxytetracycline and a bandage is highly effective, although bandaging is extremely labor intensive in herds in which the incidence of PDD is ≥5%. Rapid efficacious treatment of a large number of cattle with lesions is desired.

Footbaths containing dilute solutions of formaldehyde or copper sulfate are commonly used. However, formaldehyde solutions in footbaths pose human health hazards, are only marginally effective, and may be expensive. Other nonantibiotic solutions have had varied results. Dilute tetracycline, oxytetracycline, or lincomycin used in footbaths can control the condition. Footbaths can be difficult and expensive to manage because of the recommendation to change the solution after the passage of 300 cows.

Directly spraying effective medications on lesions is a practical option. Investigators in one study, using a pump sprayer, sprayed feet of cattle for 3 weeks to treat, control, and prevent PDD in a herd of 300 cows. With topical application, only affected cows are treated, and only those feet with lesions are treated. The purpose of the study reported here was to evaluate the risk of antibiotic residues in milk after the use of oxytetracycline solution, applied topically as a spray or in conjunction with a bandage, for treatment of cows with PDD.

Materials and Methods

Animals and procedure—Lactating Holstein cows with PDD lesions were assigned to 2 treatment groups. Treatment 1 (n = 16) consisted of application of 15 ml of a solution containing 100 mg of oxytetracycline/ml to PDD lesions. Lesions were sprayed immediately after cows were milked but before they exited the milking parlor. The solution was delivered, using a garden sprayer; lesions were sprayed twice daily for 7 days. Treatment 2 (n = 12) used bandages consisting of cotton soaked with 20 ml of a solution containing 100 mg of oxytetracycline hydrochloride/ml. The cotton was placed over the PDD lesion and held in place with tape. Cows were allowed to wear the bandage until the tape wore out and the bandage came off the foot.

Composite milk samples, consisting of an equal volume...
from each test, were obtained 5 minutes before and 24, 48, 72, 96, and 120 hours after the first spray application for cows given treatment 1; for cows given treatment 2, samples were obtained 24 and 5 hours before and 17, 48, 72, and 120 hours after application of the bandage. All milk samples were identified and frozen at -80°C. Samples were shipped on dry ice to the FDA Center for Veterinary Medicine (CVM) for analysis.

Residue testing—Analysis was performed, using high-performance liquid chromatography (HPLC) and a commercially available tetracycline screening test kit system. The HPLC procedure originally developed and validated to support a safe concentration of 30 parts/billion (ppb) of oxytetracycline in milk. Before use in this study, it was evaluated, using bulk-tank milk samples obtained from the USDA; those samples were fortified at much lower oxytetracycline concentrations (2 to 15 ppb). The standard curve was modified to include oxytetracycline concentrations of 1 to 30 ppb. Matrix interference near the retention time of oxytetracycline (in the USDA control milk samples and milk samples obtained before treatment of cows in this study) resulted in a limit of detection of 3.3 ppb of oxytetracycline in milk; this assay was used for analysis of samples obtained from cows given treatment 2. Another HPLC system and another lot of metal chelate resin were used for subsequent analyses of milk samples obtained from cows given treatment 1. There was not an obvious matrix interference for that analysis, and the limit for detection for that analysis was estimated to be <1 ppb. All samples for HPLC analysis were extracted and analyzed in duplicate.

The tetracycline screening test functions through the use of antibodies that bind to tetracycline residues in milk. The amount of tetracycline residue in the milk sample binds competitively with radiolabeled tetracycline reagent added to the milk sample. The amount of residue in the milk sample is estimated by the relative amount of radiolabeled drug that binds to the antibody in the sample, compared with the amount of radiolabeled drug that binds to the antibody in a negative-control milk sample.

The specific procedure for the tetracycline screening test kit was defined in the test kit label insert. Sensitivity of the tetracycline screening test kit was validated in an independent laboratory; 90% sensitivity was determined (with 95% confidence) to be ≥19 ppb for oxytetracycline, which was much less than the safe concentration for this drug in milk. This sensitivity provided assurance that a concentration of 19 ppb of oxytetracycline in milk will be detected by the test with a high degree of certainty.

Statistical analysis—To estimate the risk of a violative antibiotic residue, the assay result for a particular cow at a specific time can be viewed as a binary outcome with respect to whether the residue concentration is greater than or less than the established tolerance limit. These results were then used to estimate, with a specified degree of confidence, the largest percentage of cows with positive results on residue tests that would be consistent with the observed results.59

Because residue data usually have a normal distribution after logarithmic transformation, the actual HPLC results were log transformed and tested for skewness and kurtosis. The log-transformed data did not have significant skewness or kurtosis. Using procedures and principles established by the FDA-CVM,60 the residue concentration that could be expected (with 95% confidence) to be exceeded by only 1% of the population was estimated for each sample collection time.

Results

Samples obtained before treatment—Results of residue testing were recorded (Table 1). None of the samples obtained 5 minutes before cows were given treatment 1 had positive results when tested by use of HPLC. One cow in treatment 2 had detectable oxytetracycline residues (48 ppb), when tested by use of HPLC, in the sample obtained 5 hours before treatment. This sample also had positive results for the tetracycline screening test. A duplicate tube of that milk sample analyzed by use of HPLC had much less oxytetracycline (3.4 ppb), suggesting that the sample was contaminated during sample collection. None of the other milk samples obtained before treatment of the cows given treatment 2 had positive results when tested by use of the tetracycline screening test. Similarly, concentrations of oxytetracycline residues for these samples were all less than the limit of detection for the HPLC assay.

Samples obtained after treatment—There were 80 milk samples obtained after treatment from cows given treatment 1. Of these 80 samples, only 8 had oxytetracycline residues detectable by HPLC, ranging from <1 to 6.7 ppb. One of these samples had a presumptive positive result when tested by use of the tetracycline screening test. During retesting of a duplicate of that milk sample, the result was negative. Therefore, all 80 results for the tetracycline screening test were negative, and milk from all of these cows was considered negative for antibiotic residues.

Of the 48 samples obtained after treatment from cows given treatment 2, 9 had residue concentrations greater than the limit of detection for HPLC assay, with concentrations ranging from 3.5 to 12 ppb. One of these milk samples had a presumptive positive result when tested by use of the tetracycline screening test; however, during retesting of a duplicate of that sample, the result was negative. Thus, all 48 results for the screening test were negative, and milk from all of these cows was considered negative for antibiotic residues.

For treatment 1, there were 0 positive results for the 16 cows. One can be 95% confident that ≤17.1% of the cows would have antibiotic residues that were greater than tolerance limits. For treatment 2, there were 0 positive results for the 12 cows. One can be 95% confident that ≤22.1% of the cows would have antibiotic residues that were greater than tolerance limits. Because these estimates of the potential violation rate were based only on whether the assayed residue concentration was greater than or less than the tolerance limits, results would have been the same whether the tolerance limit was set at 30 or 300 ppb.

Although most of the results for the HPLC assay were less than the limit of detection, actual values for the HPLC assay were >0 ppb for samples from cows given treatment 2. The limit of detection was established as the mean + 3 SD concentration for samples obtained before treatment. Actual HPLC results can be used to establish a distribution of residue concentrations for each sample collection time. Although there were few samples with residue concentrations greater than the limit of detection, treatment 2 resulted in higher residue amounts when the residue concentrations in the samples were greater than the limit of detection. Therefore, use of the results for cows given
Table 1—Tetracycline concentrations in milk samples obtained from cows with papillomatous digital dermatitis that were treated topically, using 2 treatment methods

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>Time of sample collection</th>
<th>HPLC</th>
<th>Tetracycline screening test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not detectable</td>
<td>Greater than limit of detection†</td>
</tr>
<tr>
<td>1 (n = 16)</td>
<td>5 min before treatment</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24 h after treatment</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>48 h after treatment</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>72 h after treatment</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>96 h after treatment</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>120 h after treatment</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>2 (n = 12)</td>
<td>24 h before treatment</td>
<td>12</td>
<td>0</td>
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<tr>
<td></td>
<td>5 h before treatment</td>
<td>11</td>
<td>15</td>
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<tr>
<td></td>
<td>17 h after treatment</td>
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<td></td>
<td>120 h after treatment</td>
<td>9</td>
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</table>

*Treatment 1 = spraying of lesions with 15 ml of a solution of 100 mg of oxytetracycline/ml twice daily for 7 days. Treatment 2 = bandage consisting of cotton soaked with 20 ml of a solution containing 100 mg of oxytetracycline/ml. †Residue detected at concentration greater than the limit of detection for the assay, which was < 1.0 part/billion (ppb) for samples obtained from cows given treatment 1 and 3.3 ppb for samples obtained from cows given treatment 2. ‡Residue concentration greater than the tolerance limit (30 ppb) in effect at time study was conducted. 9May have been the result of contamination during sample collection, because a concentration of 48 ppb was detected initially, but only 3.4 ppb was detected during retesting of a duplicate sample. HPLC = High-performance liquid chromatography.

treatment 2 should result in a conservative estimate when applied to cows given treatment 1.

Assay results for samples obtained 120 hours after application of a bandage had the largest SD and resulted in the highest residue concentration that one could, with 95% confidence, be expected to be exceeded by only 1% of the population. The estimated concentration was 107 ppb for a specific cow. The FDA-CVM uses a bulk-tank factor that estimates a minimum of 10 cows contributing to the bulk tank being sampled. Incorporating this bulk-tank factor reduces the estimated residue concentration to 5.6 ppb. This means that one can be 95% confident that < 1% of the bulk-tank samples obtained would contain residue concentrations of ≥ 5.6 ppb as a result of the use of treatment 2. This value is conservative for most situations, because it is determined on the assumption that every cow in the herd would be treated at the same time.

Discussion

Current residue screening tests are developed for bulk-tank milk samples and are not approved for testing of individual cows, however, because none of the tests currently are approved for testing of samples obtained from individual cows, the bulk-tank tests commonly are used to test samples obtained from individual cows.

Testing for beta-lactam residues is required for bulk-tank milk, as indicated in Appendix N of the Pasteurized Milk Ordinance. Processors are required to use a beta-lactam test, but in addition, they may choose to use tests that detect other antibiotics.

Milk samples that have a positive result on the tetracycline screening test are considered presumptive positive for a violative residue and are retested to provide assurance that it is not a false-positive result. Retesting is performed, using the suspected milk sample (in duplicate) and positive- and negative-control samples (ie, a milk sample with a known positive result and another milk sample with a known negative result). Results for both control milk samples must provide a correct result for results of the retesting to be accepted. If either of the duplicates for the suspect milk samples has a positive result, then the milk sample will be considered positive for tetracyclines. Both of the duplicates of the suspect milk sample must have negative results to consider the milk sample negative for tetracyclines.

The study reported here provides evidence that farms that use an oxytetracycline solution topically at low doses to treat cows with PDD stand little risk of causing violative antibiotic residues. The FDA-CVM has established a tolerance limit of 300 ppb for the combined sum of residues from all tetracyclines. Other antibiotics, primarily lincomycin and spectinomycin, are used to treat cows with PDD and are used in footbaths, as topical sprays, and in bandages. Residue data on these drugs have not been established. Another remote risk for violative residues may result if a cow drinks a sufficient volume of footbath solution to cause detectable antibiotic residues. Oxytetracycline solutions used in footbaths usually have a concentration of 1.0 mg of oxytetracycline/ml, so a cow consuming 20 L (approx 5 gallons) would ingest 20,000 mg of oxytetracycline. This would most likely pose an antibiotic residue problem. If effective, use of nonantibiotic treatments may be indicated to reduce the risk of residues associated with treatment or prevention of PDD in dairy herds.

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