Persistence of Residues in Milk Following Antibiotic Treatment of Dairy Cattle

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ABSTRACT

A study was conducted to determine the persistence of antibiotic residues in milk beyond the recommended withdrawal period. Composite milk samples (n = 122) were collected from 58 lactating dairy cows in the university herd receiving antibiotic treatments for any reason but only when administered as a single drug. Samples were obtained 72 h posttreatment and sampling continued every 24 h until result for antibiotic residue was negative by the *Bacillus stearothermophilus* disc assay. The antibiotic (n = 7) administered accounted for significant variation in drug persistence; however, route of administration, case number (for cows treated for more than one episode), number of days treated, animal's body weight, lactation number, and daily milk production did not affect drug persistence. Chi-square analysis indicated that 21% of milk samples were positive for residues beyond the recommended withholding period. Milk samples from cows treated with cephapirin and penicillin were the only samples that exceeded recommended withdrawal times. Often doses administered exceeded label directions.

INTRODUCTION

Antibiotic preparations have been used to treat mastitis for nearly 30 yr (7). Although opinions differ concerning the significance of antibiotics in milk, residues are illegal (6). Residues are of concern due to their possible adverse effects on people allergic to antibiotics, the potential buildup of antibiotic-resistant organisms in humans, and inhibition of starter cultures used to produce cultured milk products such as yogurt and cheeses (5, 8, 9). Severe financial penalties imposed by milk processors, as well as lost markets and damaged reputations, are further consequences associated with antibiotic residues.

During the past few years, concern has increased over the presence of antibiotic residues in milk. In 1960, 6% of random milk samples from the United States and 4.5 to 5% of samples from England and Wales contained penicillin residues (8). Jones (4) reported residues in bulk tank milk in Virginia of .06 to .2%, which translated to a loss of approximately $580,000 annually to the Virginia dairy industry. Oliver et al. (11) found over 16% of milk samples obtained 96 h posttreatment from cows in five herds were positive for antibiotic residues using the Delvotest with residues present in 28% of cows treated via intramammary infusion and 33% of cows treated intramuscularly. Although antibiotic residues may not reduce milk sales significantly, considerable cost and effort are required from milk processors and procurement groups to ensure a safe product.

According to surveys conducted by FDA, the improper use of antibiotics to control bovine mastitis is largely responsible for the adulteration of the milk supply (12). A strict residue avoidance program is essential for prevention of residues in milk. However, even farms with the best avoidance programs may be plagued by drug residues. Mercer (9) stated that most food animal drugs approved since 1962 have undergone strict FDA testing, but many in use prior to that time have not had quality residue data developed. This category of drugs includes some of the sulfonamides, the nitrofurans, the tetracyclines, the penicillins, streptomycin, dihydrostreptomycin, neomycin, ery-

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thromycin, tylosin, bacitracin, hygromycin B, nystatin, lincomycin, spectinomycin, novobiocin, and chloramphenical (9).

Antibiotics may also be administered to dairy cattle by many different routes other than intramammary infusion. It has been suggested (1, 2) that wide variations exist between different routes of antibiotic administration and persistence of antibiotics in milk.

Objectives of this study were: 1) to determine with what frequency the milk of treated dairy cows exceeded recommended withholding periods; 2) to examine the effect of several different antibiotics on drug persistence in milk; and 3) to examine the effect of different routes of administration on drug persistence.

MATERIALS AND METHODS

Composite milk samples (equal volumes of foremilk from each quarter) were taken from 58 lactating cows in the Virginia Tech dairy herd receiving antibiotic treatment with a single drug. Milk samples were collected during the afternoon milking, beginning 72 h ± 2 h post-treatment and continuing every 24 h until a negative reading for antibiotics was observed.

Cows had been treated according to veterinarian recommendations using therapy supplied by College of Veterinary Medicine clinicians. Treatments, routes of administration, and stated withholding times are in Table 1. No withdrawal time was stated for gentamicin, and therefore, our veterinarians recommended a 7-d discard period. For cephapirin therapy, the only FDA approved intramammary treatment, our veterinarian recommended two 200 mg tubes per quarter at first treatment, followed by one tube every 24 h for an additional two treatments. Twenty-four cows were treated by this procedure. Subsequently, 10 cows with clinical mastitis or elevated DHIA SCC were treated according to label recommendations using two cephapirin treatments but at 24-h interval rather than 12 h. All treatments were administered once daily by the herd manager. Milking times are 0030 and 1230 h. Veterinarian-recommended dose of penicillin was approximately 2 ml/45.5 kg body weight or twice label directions.

Milk samples were analyzed by the *Bacillus stearothermophilus* disc assay (10, 13, 17). Details of this procedure were reported by Seymour et al. (16). Zones of >14 mm indicated the presence of inhibitory residues, and zones of ≤14 mm were considered negative for residues (6).

Statistical Analyses

Data were analyzed by the General Linear Model procedure of SAS (15). The following model was used:

$$Y_{ijklmno} = \mu + D_i + R_j + C_k + L_l + B_m + N_n + b_1 M_o + E_{ijklmno}$$

where $Y_{ijklmno}$, the dependent variable, was days of persistence minus the stated withholding time. A negative value or zero would indicate that milk was free of antibiotic contamination on or before the end of the stated withholding period. A positive value would indicate that milk was contaminated with antibiotics beyond the recommended withholding time. Other variables in the model were μ, mean days of persistence; D_i, effect of drug (i = 1, 7); R_j, effect of route of administration (j = 1, 5); C_k, effect of case number (for cows treated for more than one episode) (k = 1, 3); L_l, effect of length of time treated (days) (1 = 1, 7); B_m, effect of body weight (three weight categories: <500 kg, 500 to 700 kg, and >700 kg) (m = 1, 3); N_n, effect of lactation number (n = 1, 8); M_o, effect of milk production of the o^{th} individual (kg/d); b_1 the regression of milk yield on Y; and $E_{ijklmno}$, the residual error. Chi-square analysis was conducted to compare results for the percent matching the recommended withdrawal time.

RESULTS AND DISCUSSION

The antibiotic administered to the animal was the only variable in the model that ($P<.05$) affected drug persistence in milk. Neither route of administration, case number (for cows treated for more than one episode), number of days treated, animal's body weight, lactation number, nor daily milk production contributed significant variation to the model. Route of administration was not related to drug persistence, although other studies showed different routes caused variation in persistence of antibiotics in milk (1, 2). Only two of the eight

drugs administered, each by a different route of administration, exceeded the recommended withdrawal periods. However, 67% of all cows treated received one or the other of these two treatments. Cannon et al. (2) observed that neither average daily milk production, fat percentage in milk, nor body weight contributed significantly to variation in the excretion of penicillin in milk, which supports results of this study.

Table 1 presents the distribution of days of persistence minus the recommended withholding time for each individual drug administered during this study. Milk from cows treated with cephapirin (intramammary) and penicillin (intramuscular) was positive for days persistence, indicating contamination beyond the recommended withholding period. Of the 24 cows treated with cephapirin, 8 (33%) exceeded the stated withholding time of 96 h with 3 exceeding it by 72 h. Because the cephapirin product was not administered according to manufacturer's directions, 10 additional cows from the Virginia Tech herd

TABLE 1. Distribution of days of persistence minus the recommended withholding time for each drug administered during this study.

Drug and route	Withholding period (days)	Source	n[1]	Days[2]	Percent
Cephapirin,[3] intramammary	4	Label	24	−1	58.3
				0	8.3
				1	12.5
				2	8.3
				3	12.5
Penicillin,[4] intramuscular intrauterine	3	Label	15	0	73.3
				1	6.7
				2	6.7
				3	6.7
				6	6.7
Gentamicin,[5] intramammary	7	Vet[10]	3	−4	66.7
				−3	33.3
Polymyxin B,[6] intramammary	4	Vet[10]	1	−1	100.0
Liquamycin,[7] intravenous	4	Vet[10]	7	−1	100.0
Naquasone,[8] oral	3	Label	4	0	100.0
Triple sulfa,[9] oral	4	Label	4	−1	100.0

[1] Number of cows treated with each drug.

[2] Days of persistence minus the recommended withholding time.

[3] Cefa-Lak, Sodium Cephapirin (200 mg/tube), Bristol Laboratories, Syracuse, NY.

[4] Procaine penicillin G (300,000 units/ml, 2 ml/45.4 kg body weight), Pfizer, New York, NY.

[5] Gentocin (100 mg gentamicin sulfate/ml, 1 to 5 ml/treatment), Schering Corp., Kenilworth, NJ.

[6] Polymyxin B Sulfate (250 to 500,000 units/dose), The Upjohn Co., Kalamazoo, MI 49001.

[7] Liquamycin 100 (100 mg oxytetracycline hydrochloride/ml, 60 ml/treatment), Pfizer, New York, NY. Label reads "Not for use in lactating dairy animals."

[8] Naquasone (200 mg trichlormethiazide plus 5 mg dexamethasone), Schering Corp., Kenilworth, NJ.

[9] Triple Sulfa Injectable 24% (370 grains sulfonamides/100 ml, 200 ml dose), Tech America Group Inc., Elwood, KS.

[10] Veterinarian recommendation.

TABLE 2. Distribution of days of persistence minus the recommended withholding time for cows treated with cephapirin according to label directions.

Drug	Withholding period (d)	n[1]	Days[2]	Percent
Cephapirin	4	10	−1	50.0
			0	10.0
			1	20.0
			2	10.0
			3	10.0

[1] Number of cows treated.

[2] Days of persistence minus the recommended withholding time.

were treated according to label directions and 4 (40%) exceeded the recommended withholding period of 96 h (Table 2). These results were similar to those obtained with the elevated dosage of cephapirin recommended by our veterinarian. Of the 15 cows treated with penicillin, 4 (27%) exceeded the withdrawal time, and 7% (one cow) did so by 6 d.

Mercer (9) stated that most food animal drugs approved before 1962 have not had definitive data on residue withdrawal times developed; this included the penicillins. This may be the reason for the persistence of this drug beyond its specified withdrawal time. Another possibility is that the withdrawal period is set for a dosage of 1 ml/45.4 kg of body weight, but in actual practice a much higher dose is often administered (approximately twice this dosage). The label indicates that treatments exceeding 3000 units/.454 kg body weight or greater than 10 ml/injection site may cause residues in milk beyond the withdrawal time. Each injection approximated 13 ml or 6000 units/.454 kg body weight.

The reason for the persistence of cephapirin residues beyond the withholding period is unclear. The product was approved for use in lactating dairy cattle in 1975, so that definitive data on residue withdrawal times have been developed (9). Testing methods have increased in sensitivity since that time and may be an answer to problems with extended withdrawal. The *B. stearothermophilus* disc assay, with a sensitivity of .005 to .008 IU penicillin (including cephapirin)/ml, was accepted by the AOAC in 1979 (12), 4 yr after approval of the cephapirin preparation. Prior to 1979, the *Bacillus subtilis* assay, with a sensitivity of .05 IU penicillin/ml, and the *Sarcina lutea* assay, with a sensitivity of .01 IU penicillin/ml, were the most commonly used official methods for residue detection (12). Differences in sensitivity between the *B. stearothermophilus* assay and previous assays may explain the discrepancy. Another explanation may be that the *Code of Federal Regulations* (3) has set a legal tolerance for cephapirin in milk of .02 μg or units/ml but the *B. stearothermophilus* assay may detect .0030 to .0059 μg/ml (14).

Chi-square analysis was conducted to compare sample results for percent matching the recommended withdrawal time (Figure 1). Results indicate that 79% of cows produced milk with no contamination beyond the withholding period. Conversely, 21% of these cows produced contaminated milk for varying

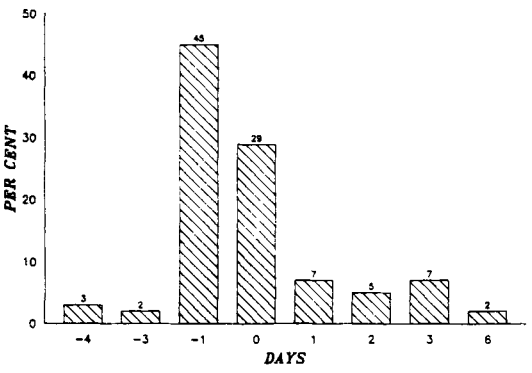

Figure 1. Distribution of days of persistence minus the recommended withholding time for cows treated during this study.

periods beyond the recommended discard time, but several antibiotics were administered at dosages exceeding label recommendations. However, cows receiving intramammary cephapirin treatments at recommended dosage showed residue persistence similar to that of cows treated with higher dosage. Larocque and Neville (7) suggested that most of the currently available antibiotic intramammary products were developed and introduced to the market many years ago, before comprehensive and specific testing methods were developed. Also, Figure 1 shows that 50% of cows produced milk clear of antibiotics by 24 h before the end of the recommended withholding period. Our data indicate that antibiotic residues may be present in milk at the end of the recommended withdrawal time when therapy is administered in excess of label directions. We suggest that milk of antibiotic-treated cows should be monitored by an antibiotic residue test before milk from these cows is added to the milk supply.

REFERENCES

1 Albright, J. L., S. L. Tuckey, and G. T. Woods. 1961. Antibiotics in milk – a review. J. Dairy Sci. 44:779.

2 Cannon, R. Y., G. E. Hawkins, and A. M. Wiggons. 1962. Duration of secretion of bacteriostatic drugs in milk. I. Penicillin, following oral and parenteral administration. J. Dairy Sci. 45:769.

3 Code of Federal Regulations. 1987. Food and drugs. Pages 460–486 *in* 21 CFR 556.115. US Govt. Printing Office, Washington, DC.

4 Jones, G. M. 1982. Your processor might not want your milk. Hoard's Dairyman 127:1120.

5 Jones, G. M., and E. H. Seymour. 1988. Cowside antibiotic residue testing. J. Dairy Sci. 71:1691.

6 Katz, S. E. 1982. General referee reports: Committee G. Report on antibiotics. J. Assoc. Offic. Anal. Chem. 65:358.

7 Larocque, L., and G. A. Neville. 1985. Quantitative evaluation of a bovine antibiotic infusion product by milk residue depletion studies. J. Food Prot. 48:611.

8 Lindemayr, H., R. Knobler, D. Kraft, and W. Baumgartner. 1981. Challenge of penicillin-allergic volunteers with penicillin-contaminated meat. Allergy 36:471.

9 Mercer, H. D. 1975. Antimicrobial drugs in food-producing animals. Vet. Clin. North Am. 5:3.

10 Messer, J. W., J. E. Leslie, G. A. Houghtby, J. T. Peeler, and J. E. Barnett. 1982. *Bacillus stearothermophilus* disc assay for detection of inhibitors in milk: collaborative study. J. Assoc. Offic. Anal. Chem. 65:1208.

11 Oliver, S. P., R. T. Duby, J. P. Tritschler II, and R. W. Prange. 1984. Residues in dairy cows following antibiotic administration. Page 125 *in* Proc. 23rd Annu. Mtg. Natl. Mastitis Counc., Arlington, VA.

12 Ouderkirk, L. A. 1979. *Bacillus stearothermophilus* disc assay for detection of residual penicillins in milk: collaborative study. J. Assoc. Offic. Anal. Chem. 62:985.

13 Peeler, J. T., J. E. Leslie, J. E. Barnett, G. A. Houghtby, and J. W. Messer. 1983. Detectability and precision of the AOAC *Bacillus stearothermophilus* disc assay demonstrated in the 1981 FDA split milk sample testing program. J. Food Prot. 46:84.

14 Rajkowski, K. T., J. T. Peeler, and J. W. Messer. 1986. Detectability levels of four beta-lactam antibiotics in eight milk products using the AOAC *Bacillus stearothermophilus* disc assay. J. Food Prot. 49:687.

15 SAS Institute, Inc. 1982. SAS User's guide: statistics. Cary, NC.

16 Seymour, E. H., G. M. Jones, and M. L. McGilliard. 1988. Comparison of on-farm screening tests for detection of antibiotic residues. J. Dairy Sci. 71:539.

17 US Department of Health and Human Services. 1985. Grade A pasteurized milk ordinance. Publ. No. 229. Rockville, MD.