

Retention Data for Antibiotics Commonly Used for Bovine Infections¹

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ABSTRACT

This study was designed to observe the maximum time that various antibiotics used in different ways as treatment of bovine infections persisted in milk after final treatment. Both Delvotest-P and *Bacillus stearothermophilus* (Difco) disc assay procedures were utilized for detection of antibiotic preparations used for treatment of mastitis. None persisted in milk longer than specified on their respective labels. Because antibiotic residues were detected in milk consequent to treatment for intrauterine infections, guidelines for withholding times following intrauterine treatment should be established.

INTRODUCTION

Antibiotics have been used in dairy cattle management for more than three decades. They are administered to cattle by any four general methods: 1) infusion into the udder for treatment of mastitis; 2) injection (intramuscular, intravenous, or subcutaneous) for treatment of numerous diseases; 3) orally for treatment of diseases or as a dietary supplement; and 4) reproductive "flush" for uterine, cervical, and vaginal infections. Such uses have led to contamination of milk and milk products with antibiotics. Use of infusible antibiotic products for treatment of mastitis in cows is a public health concern because such products sometimes are not used according to label directions. Improper milk discard times and multiple dosing with the same or another product can create drug residue problems (3). In addition,

residues can occur after proper adherence to withdrawal times, but occurrence of residues from this cause is relatively rare (4). The Food and Drug Administration (FDA) considers antibiotic-contaminated milk adulterated. Such adulteration can be minimized by exclusion of contaminated milk from the general milk supply. The FDA has attempted to reduce adulteration by limiting the quantity of antibiotic in each preparation to be used for mastitis therapy and by a requirement that a warning against the use of milk from recently treated animals be placed on the preparation (6).

Antibiotic residues in milk should be avoided because of three distinct concerns: residues are illegal; the milk is from treated cows and may contain large numbers of potential pathogens; and there may be biologically active metabolites of drugs in the milk that could result in anaphylaxis (4). In addition, antibiotics in milk in even minute quantities have created several problems in dairy processing and quality control including: inadequate curdling of milk and improper ripening of cheeses during their manufacture; decreased acid and flavor production during the manufacture of buttermilk and similar products; diminished starter culture growth propagated in reconstituted skim milk; and validity of certain quality control tests (7). These can result in inferior products that must be discarded, causing costly equipment cleanup and subsequent disruption of processing schedules. The objective of this study was to observe retention times of antibiotic residues in milk from lactating dairy animals that had been treated for infection.

MATERIALS AND METHODS

The two antibiotic detection assays were the Delvotest-P and Difco disc assay. The reason for these procedures was their relative rapidity, sensitivity, and simplicity.

The Delvotest-P (8) is an agar diffusion test. Tablets containing nutrients and bromocresol

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purple are added to ampules containing plain agar with spores of *Bacillus stearothermophilus* var. *calidolactis*. After .1 ml of milk sample is added, the ampule is incubated 2.5 h at 63 to 66°C. In the absence of antimicrobial substances, the whole of the solid medium turns yellow (negative result), whereas it remains purple in the presence of sufficiently high concentrations of antibiotics (positive result). At intermediate concentrations of antibiotic the solid medium turns partly yellow (doubtful result). The test method has a high sensitivity for penicillin. Concentrations of .002 units/ml or less give negative results in all samples, and .003, .004, and .005 units/ml give predominantly negative, doubtful, and positive results, respectively. These results are in bulk milk samples and in individual milk samples. The method is also sufficiently sensitive to most antibiotics usually administered to lactating cows (8).

The Difco disc assay (5) employs Antibiotic Medium #4 (Difco) seeded with *Bacillus stearothermophilus* (Difco). Six milliliters of the seeded agar are put into each 15 × 100 mm plastic petri dish (Fisher Scientific). Plates must be used within 7 days of date prepared. Thirteen millimeter paper discs (Schleicher and Schuell) are soaked with the milk samples by capillary action and placed on the agar. The plate is incubated for 2.5 h at 64°C. An alternate method is for incubation to proceed for 4 h at 55°C. After incubation is complete, zones of inhibition then are measured in millimeters. A positive result is designated by a zone of 15 mm, which is a compromise between 14 or 16-mm zones suggested for a positive result (1, 2). This assay detects as little as .005 units penicillin/ml of milk. Both assays were conducted each time a milk sample was analyzed for antibiotic residues.

Objective was to observe retention times of selected antibiotics for cows treated for infection. The Clemson University LaMaster Dairy Center herd, which consists of approximately 105 Holsteins, 26 Guernseys, and 31 Jerseys, was utilized for this phase, which was undertaken in four parts. Cows diagnosed with clinical mastitis as determined by abnormal milk such as discoloration and flakes were treated by one of three procedures. The first mastitis treatment was intramammary infusion with Forte® (The Upjohn Co.), an antibiotic preparation containing procaine penicillin and

sodium novobiocin (Table 1). Each animal was treated with the 10-ml preparation in the infected quarter immediately following the milking during which infection was discovered and then again 24 h later as suggested by the manufacturer. Milk samples were collected each milking in sterile "Whirl" bags (Nasco) after the initial treatment. Seven Holsteins, four Guernseys, and four Jerseys were treated in this manner, and milk samples were tested for antibiotic residues.

The second mastitis treatment was intramammary infusion with Today® (Bristol-Myers Co.), an antibiotic preparation of sodium cephalirin (a cephalosporin) (Table 2). Each cow was treated with the 10-ml preparation in the infected quarter immediately following the milking during which infection was discovered and then again 12 h later as suggested by the manufacturer. Milk samples were collected each milking in "Whirl" bags after the initial treatment. Due to limited incidence of mastitis, only five Holsteins were treated in this manner and milk samples tested for antibiotic residues.

The final mastitis treatment was intramuscular injection with Combiotic® (Pfizer, Inc.), an antibiotic preparation of procaine penicillin and dihydrostreptomycin sulfate (Table 3). Each cow was treated with 15 ml in the hip muscle immediately following the milking during which infection was discovered and then again 24 h later as suggested by the manufacturer. Milk samples were collected as described. Because of limited incidence of mastitis, only four Holsteins were treated in this manner, and milk samples tested for antibiotic residues.

For treatment of intrauterine infection, as diagnosed by the herd manager responsible for artificial insemination, each animal was infused

TABLE 1. Maximum retention times of antibiotic residues in milk after the final intramammary infusion of Forte®.

Breed	Cows	Retention times	SE
	(n)	(h)	
Holstein	7	30.9	4.4
Guernsey	4	27.0	5.8
Jersey	4	33.0	5.8

TABLE 2. Maximum retention times of antibiotic residues in milk after the final intramammary infusion of Today®.

	Animals					\bar{X}	SE
	1	2	3	4	5		
Retention time (h)	60	72	24	48	48	50.4	8.0

TABLE 3. Maximum retention times of antibiotic residues in milk after final intramuscular injection of Combiotic®.

	Animals					\bar{X}	SE
	1	2	3	4	5		
Retention time (h)	36	48	60	36	45.0	5.7	

with 10 ml Combiotic® following morning milking, then again 48 h later (Table 4). Milk samples were collected as described. Nine Holsteins, five Guernseys, and four Jerseys were treated in this manner, and milk samples were tested for antibiotic residues.

For each of the four treatments, milk samples were collected and tested for antibiotic residues until two consecutive samples assayed negative. Experimental designs were randomized completely for each of the four treatments with means and standard errors calculated and compared to maximum retention times of antibiotic residues as specified on the label of each antibiotic preparation. For both the intramammary Forte® infusion and the intrauterine Combiotic® infusion, variance was analyzed and the F-test used to detect any significant breed variation for antibiotic retention between Holsteins, Guernseys, and Jerseys. This breed difference may be a factor in residue retention as milk is the major excretory route of antibiotic residues, and Guernseys and Jerseys generally produce significantly less milk than Holsteins.

RESULTS AND DISCUSSION

Maximum retention times of antibiotic residues in milk after final intramammary

infusion of Forte® for treatment of mastitis proved to be less than the 72 h withholding time specified for the product (Table 1).

No effect of breed was detected on retention times of antibiotic residues in milk. This possibly could be accounted for by the theory that even though milk production among the three breeds is generally different, a large percentage of the infused antibiotic is bound to the organism(s) involved as a result of "combating" the infection. Therefore, even though there may be less of a carrier medium available for excretion of the antibiotic, there may not be a concentrating effect because there may be less free antibiotic to be excreted in quantities detectable by assays.

Antibiotic residues were detected in milk of cows treated for intrauterine infections by infusion of Combiotic® (Table 4).

Because there is no approved withholding time of milk from animals treated with antibiotics for intrauterine infections, that antibiotic residues are in milk after such treatment is important. No differences were between breeds even though retention time of Jerseys appeared different. This is probably due to the wide range and small number of animals.

TABLE 4. Maximum retention times of antibiotic residues in milk after final intrauterine infusion of Combiotic®.

Breed	n	Average retention time	Range	SE
		(h)		
Holstein	9	9.3	0 - 24	4.2
Guernsey	5	12.0	0 - 24	5.6
Jersey	4	21.0	0 - 48	6.3

CONCLUSIONS

Cows that had contracted mastitis and were treated with an antibiotic preparation did not retain antibiotic residues in milk longer than the maximum withholding time specified by the manufacturer of the product. Cows that had contracted a uterine, cervical, or vaginal infection and were treated by intrauterine infusion with an antibiotic preparation produced milk containing detectable antibiotic residues in their milk. Guidelines should be established for maximum withholding times of milk from cows treated in this manner.

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