Effect of Antibiotic Administration on Mastitis Screening Tests and Milk Composition

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Abstract
Intramammary infusion of a commercial antibiotic mixture or aqueous penicillin resulted in a marked inflammatory reaction characterized by increases in leucocyte numbers and degree of reaction to mastitis screening tests. Milk lactose content was decreased significantly. Penicillin infusion resulted in an apparent decrease in streptococcus organisms, but had little effect on staphylococci.

Intramuscular injection of streptomycin had only minor effects on leucocytes, screening tests, milk composition, or bacteriological status of the individual quarters.

Intramuscular penicillin injection resulted in significant decreases in leucocytes and reactions to the mastitis screening tests. Protein and lactose content of the milk were increased. There was a reduction in the number of quarters shedding staphylococcus as well as streptococcus organisms.

Increased use of mastitis screening tests on bulk milk by milk plants and regulatory agencies has resulted in increased use of the California Mastitis Test (CMT) on individual quarters. There is a tendency to consider antibiotic treatment of quarters positive to the CMT, even though these positive quarters may not show visibly abnormal milk or clinical mastitis.

Garrison and Turner (4) reported changes in milk composition and increased leucocytes as a result of infusing up to 900 ml of distilled water per quarter; however, when the level was decreased to 250 ml, there was little change in milk composition.

Several authors (5, 6, 10) have shown that the use of intramuscular or intramammary treatment reduced the number of quarters shedding streptococcus organisms. These reports did not indicate what effect the treatments had on the mastitis screening test reaction or milk composition.

The purpose of this study was to determine the effect of various antibiotic treatments, administered by intramammary or intramuscular routes, upon mastitis screening test reactions as well as milk composition. The intent was to study cows with varying initial reactions to the screening tests, but not showing visibly abnormal milk or clinical mastitis.

Experimental Procedure
Four trials involving eight cows each were conducted. Previous CMT reactions of the individual quarters were used in selecting cows, to obtain a range in reaction from negative to positive. All quarters were negative to the strip-cup test before treatment. A quarter milker was used for sampling at one milking each day, with a regular milker being used at the opposite milking. Values from the first two days were averaged and used as the control. Treatment followed the final milking of the control period. Sampling was continued for three days after treatment, with a final sample taken 1 wk after treatment to determine if a change from the control level had occurred.

Milk fat was determined by the Babcock method. Lactose was measured by the Benedict method (3). The Orange G dye-binding procedure was used to determine protein (12). The CMT (9) was recorded as 0, 1, 2, or 3, with 0 and 1 being considered negative, and 2 and 3 positive. The catalase test as described by Spencer and Simon (11) was used, with slight modification. Chlorides were determined by the method described by Nageswararao and Blobel (7), using the Buchler Cotlove chloridometer. The Levowitz and Weber (2) stain was used in the preparation of slides for leucocyte counts. Twenty-five fields were counted at random per slide. Smears of two different sizes were used to allow more accuracy in counting, with a wide range in leucocyte concentration.

The following treatments were given: 1) intramammary infusion of a commercial antibiotic mixture (28 ml); 2) intramammary infusion of 250,000 units of penicillin G potassium per

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MASTITIS SCREENING TEST

quarter in 20 ml of pyrogen-free saline; 3) intramuscular injection of 1 g of aqueous dihydrostreptomycin sulfate per 68.1 kg of body weight; 4) intramuscular injection of 6,000 units of procaine penicillin G per .45 kg of body weight. All treatments were given as a single dose.

After the skin on the teat ends had been washed with 70% alcohol, milk samples were drawn for bacteriological examination. The samples were then sent to the Wisconsin Central Animal Health Laboratory for isolation and identification of organisms on bovine blood agar. One set of samples was taken on the first day of the trial, and a second 1 wk after treatment, for bacteriological examination.

In an attempt to express the type of change which occurred due to treatment, a regression model including the linear, quadratic, and cubic terms was used. Results of Days 2, 3, 4, 5, and 9 were compared to their linear, quadratic, and cubic regressions, to determine significant trends.

Results and Discussion

Intramammary commercial. In Trial 1, all four quarters of each of the eight cows were treated with the commercial antibiotic mixture routinely used in the University herd. The skin at the end of the teats was washed with 70% alcohol before infusion. The quarters ranged from negative to strongly positive in CMT reactions before treatment. The effect on milk composition is shown in Figure 1. Day 0 represents the average of the two-day control period. Lactose decreased from a control value of 4.47 to 3.92% one day after treatment, but exceeded the control level by the end of the trial. Protein percentage increased gradually throughout the treatment period. Milk production increased on Day 3, and decreased below the control level 1 wk after treatment. Milk fat increased markedly on Day 1 after treatment and remained above the control levels throughout the trial. A portion of the initial increase may be accounted for by the oil base of the treatment mixture, which was picked up in the fat column. Changes in milk composition were consistent with those found under mastitic conditions (8). The effect of treatment on mastitis screening tests is shown in Figure 2. There was a large inflammatory reaction, as shown by the screening test results. Catalase values increased from 34 to 96% by Day 2 after treatment, and returned to control levels within 1 wk after treatment. Leucocytes increased markedly before returning to normal 1 wk after treatment. The CMT increased from an average of 1.47 to a maximum of 3 for two days after treatment, before decreasing to the control level at the end of the trial. Chloride increased from .1371 to .1659% as a result of the treatment. On Day 2 after treatment, 17 of the 32 quarters were exhibiting abnormal milk by the strip-cup test. These data indicate that the inflammatory re-

![Figure 1. Effect of intramammary infusion of a commercial antibiotic mix on milk composition.](image1)

![Figure 2. Effect of intramammary infusion of a commercial antibiotic mix on mastitis screening tests.](image2)
Intramammary penicillin. Penicillin in a pyrogen-free saline solution was used in Trial 2, to determine if the prolonged inflammatory reaction had been due to the oil base used in the previous trial. All quarters of the eight cows were treated, using individual sterilized teat cannulae. The skin at the end of the teats was washed with 70% alcohol before infusion. Results are summarized in Figure 3. Lactose percentage decreased on Day 1 after treatment, but returned to normal two days later. Protein increased and remained above the control levels throughout the trial. Fat percentage increased with a corresponding decrease in milk production on the first day after treatment, but returned to control levels by the end of the week. As in Trial 1, a large inflammatory response was noted. However, most of the mastitis screening test scores had returned to normal by three days after treatment. As shown in Figure 4, catalase values increased from 45 to 94%, and returned to control levels in three days. Increases of 3.2, .9, and .017 were noted in leucocytes (mil/ml), CMT, and chloride per cent, respectively, before returning to control levels. Significant changes (P < .01) in all constituents except protein were elicited by treatment. Results of the bacteriological data are summarized in Table 1. Intramammary infusion of penicillin had little effect in reducing the number of quarters shedding staphylococci, but was effective in reducing the number of quarters shedding streptococci. A high incidence of new quarters shedding organisms was noted. This

![Graph](image)

**Fig. 3.** Effect of intramammary infusion of penicillin on milk composition.

**Fig. 4.** Effect of intramammary infusion of penicillin on mastitis screening tests.

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<th>TABLE 1</th>
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<td><strong>Effect of mastitis treatments on microorganisms</strong></td>
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<td>New</td>
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<td>H. Strept.</td>
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<td>S. Strept.</td>
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<td>New</td>
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<td>Negative</td>
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Number of quarters shedding organisms: * before treatment; † remaining after treatment; ‡ new infections.
may be due to removal of teat canal keratin by introducing a tube into the teat canal during treatment (1, 6).

From results of Trials 1 and 2, it would appear that intramammary infusion was not effective in reducing the mastitis screening test reactions when used on quarters which were negative or had subclinical mastitis. There was a marked increase in screening test reactions on Days 1 and 2 after treatment, with decreases to levels approximating the initial values 1 wk after treatment. The decrease in lactose per cent as a result of intramammary infusions was similar to results of Garrison and Turner (4). They postulated that the reduced lactose synthesis was caused by increased entrance of chloride into the milk as a result of increased tissue permeability. Lactose synthesis by the cells was decreased to maintain the osmotic pressure equilibrium with the blood. Wheelock and Rook (13) observed lactose in the blood and urine of cows on which several milkings had been omitted. The decrease in lactose obtained in these trials may have been a result of lactose leaving the udder and entering the blood.

The increased protein per cent appears to have been a result of whey proteins entering the milk from the blood (4).

**Intramuscular streptomycin.** In Trial 3, a single large dose of streptomycin was injected intramuscularly after the final milking of the control period. Results of Trial 3 are shown in Figures 5 and 6. Lactose percentage was significantly higher (1% level) than the initial level 1 wk after treatment. No significant changes in fat, protein, or milk production were found. Catalase values decreased toward the end of the trial. This change was significant at the 1% level. Treatment had very little effect on leucocyte numbers, CMT reaction, or chloride content. Table 1 indicates that streptomycin was ineffective in reducing the number of quarters shedding organisms.

**Intramuscular penicillin.** The effect of a large single dose of intramuscular penicillin on milk composition is shown in Figure 7. Lactose decreased significantly (1% level) from a control value of 4.76 to 4.32% two days after treatment, but increased to a trial high of 4.99% 1 wk after treatment. Protein and fat percentages increased significantly (1% level) during the treatment period. Milk production was variable during the trial. Figure 8 summarizes the effect on mastitis screening tests. Significant decreases (1% level) of .012, .47, and 9.1 in chloride per cent, CMT, and catalase (O_2 per cent), respectively, occurred when comparing the control average to the values 1 wk after treatment. The number of leucocytes decreased by .5 mil/milliliter as a result of treatment, this change being significant at the 5% level.

The bacteriological data in Table 1 indicate that intramuscular injection of penicillin was effective in reducing the number of quarters shedding organisms. Seventeen quarters were shedding hemolytic staphylocoeci during the
control period, but only five quarters were still shedding them at the end of the trial. The three quarters shedding hemolytic streptococci at the start of the trial were negative at the end of the trial. One of the new quarters showing hemolytic streptococci at the end of the trial was a quarter in which a teat dilator had been used during the trial. The number of negative quarters increased from 12 to 20 from the beginning to the end of the treatment period.

These results show that intramuscular penicillin was effective in increasing protein and lactose percentage, decreasing mastitis screening test reactions, and reducing the number of quarters shedding organisms.

References