Efficacy of Two Therapy Regimens for Treatment of Experimentally Induced Escherichia coli Mastitis in Cows

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ABSTRACT

The objective of the study was to monitor the effect of two therapy regimens on experimental Escherichia coli mastitis. Single udder quarters of 12 cows that were at least 30 d postpartum were inoculated with 1500 cfu of E. coli. The inoculation was repeated in the contralateral quarter after a 3- to 4-wk interval. Initially, half of the cows were treated with antimicrobials, and the remaining half were left untreated. At the second inoculation, the cows that were originally treated were not treated, and vice versa.

Therapy began 12 h after inoculation and consisted of parenteral trimethoprim-sulfadiazine (6 cows) or intramammary colistin sulfate (6 cows). Clinical signs, daily milk yield, bacterial count, and endotoxin content of the milk were recorded. Milk SCC, NAGase activity, and trypsin inhibitor capacity were also monitored.

The response to bacterial challenge varied greatly among cows. Bacteria were eliminated from the quarters within 7 d in all but 1 cow. Treatment did not significantly affect the elimination rate of bacteria or any of the measured parameters. Significant positive correlations existed among milk bacterial counts, endotoxin concentrations, and clinical signs at the acute stage of the infection. Based on these findings, antimicrobial therapy of E. coli mastitis during lactation apparently is no more beneficial than no treatment. (Key words: mastitis, therapy, Escherichia coli)

Abbreviation key: LAL = limulus amoebocyte lysate, MIC = minimum inhibitory concentration, TIC = trypsin inhibitory capacity, TMP-S = trimethoprim-sulfadiazine.

INTRODUCTION

Coliform mastitis is often associated with severe systemic and local signs and causes considerable losses in milk yield. Despite the frequent occurrence of the disease worldwide, an efficient therapy has not yet been established unequivocally. The signs in coliform mastitis are known to be due to production of lipopolysaccharide endotoxin, and spontaneous elimination of bacteria from the quarter is high (1, 4, 7, 14). Antimicrobial agents continue to be routinely administered for treatment of coliform mastitis despite a paucity of evidence for their therapeutic value. Administration of some antibiotics that were effective in vitro did not improve clinical recovery or elimination of bacteria in cases of spontaneous or experimentally induced coliform mastitis (4, 10, 18).

For cattle, only a few antibiotics are registered as being effective against Gram-negative organisms (4). Some drugs are available for intramammary use, but distribution of the drugs in the swollen udder quarter is limited. In addition, high concentrations of certain antibiotics in the milk compartment may interfere with phagocytosis (24), which is of major importance in controlling coliform infections. Antimicrobials from the polymyxin group inactivate endotoxin in vitro (23), but their ef-
ficacy in vivo is not known. In systemic therapy of lactating cows, the pharmacokinetics of most antimicrobials that are effective against Gram-negative bacteria is unfavorable (4, 16). Residues of many drugs, in particular those resulting from extralabel use, can cause problems (1, 4).

This study examined the effect of two antibacterial therapy regimens on experimentally induced E. coli mastitis. The combination trimethoprim-sulfonamide (TMP-S) was selected because it is one the drug therapies of choice for coliform mastitis in Europe. The second drug tested was colistin, which is reported to neutralize endotoxins in milk (23).

MATERIALS AND METHODS

Experimental Cows
Twelve clinically healthy, multiparous Finnish Ayrshire cows were used in the experiments. The cows had been lactating for at least 1 mo, and most were in late lactation; the mean was 210 d of lactation. The mean yield was 16.5 L of milk/d (range, 11 to 26 L). Cows were housed in a stanchion barn, milked twice daily, and fed a diet of good quality silage, hay, and grain. Water was provided for ad libitum intake. One to 2 wk before the experiment began, the udders of the cows were examined clinically, and milk samples were taken for bacteriological culture, milk SCC, and NAGase activity determination. This procedure was repeated 2 d before challenge. All the cows had a low SCC (<250,000 cells/ml) in bulk milk. The experimental and control quarters used in the study were culture-negative and had milk SCC of <150,000/ml and NAGase activities of <10 AU (arbitrary units) (15).

Intramammary Challenge
One quarter of each cow was challenged intracisternally with Escherichia coli (strain FT238) isolated from a clinical case of mastitis. The strain was nonhemolytic, intermediate resistant to serum (3), and sensitive in vitro to TMP-S [minimum inhibitory capacity (MIC); <1.2 μg/ml] and colistin (MIC <8 μg/ml). The strain was stored at -70°C until used. When needed, the strain was grown on blood agar plates. A few colonies were subcultured into ISB broth (Oxoid, Basingstoke, Hampshire, England) and incubated for 18 h at 37°C. The broth culture was centrifuged at 4000 rpm for 10 min, and the pellet was resuspended in sterile saline. The bacteria were washed three times in saline and diluted to an estimated concentration of 10^8 cfu/ml. The final concentration was determined by measuring the turbidity at 620 nm. The inoculation dose used was approximately 1500 cfu/ml infused in 5 ml of pyrogen-free saline. The actual bacterial counts of the final inoculum were determined by the plate count method; the counts ranged from 1.4 x 10^3 to 2.1 x 10^3 cfu/ml. Teats were challenged using a blunt cannula via the teat orifice after careful disinfection of teat ends with 70% alcohol. The cows were inoculated twice in hind or front quarters, the second inoculation in the contralateral quarter of each cow being made after an interval of 3 to 4 wk from the first challenge. Inoculations were done 2 h after morning milking.

Treatment
At the initial challenge, half of the cows were treated with antimicrobials, and the other half were left untreated. At the second challenge, those cows originally treated were left untreated, and those originally untreated were treated. Cows were randomly allocated to the two treatment groups. Two antimicrobial therapy regimens were used: 1) TMP-S (Tribrissen®, Coopers Tierarzneimittel GmbH, Friesoythe, Germany) at 48 mg/kg, first intravenously and then intramuscularly, three times at 12-h intervals (6 challenges); and 2) colistin sulfate (Polymast®, Provelux, Luxembourg), at 6 million units intramammarily, three times at 12-h intervals (6 challenges). The intramammary product also contains 300 mg of oleandomycin phosphate, which has no activity against Gram-negative agents in vitro. Treatment started 12 h after challenge when clinical signs became evident.

Clinical Observations
Systemic and local clinical signs were monitored throughout the experiment. Heart rate and rectal temperature were determined every 2 h after challenge during the first 8 h and thereafter twice a day for 2 d. Rumen
motility was evaluated during the same period. Systemic signs were scored on a three-point scale (1 = no signs to 3 = severe signs). Clinical status of the inoculated and control quarters was recorded at the time quarter milk samples were taken. Clinical status was recorded on a three-point scale (1 = no changes in the udder quarter to 3 = severe swelling and soreness in the quarter). A similar scoring system was used to evaluate milk appearance (1 = normal milk to 3 = serous or clotty milk) (17).

Total milk yield was measured before and after challenge, during the 1st wk every other day and thereafter once a week for 3 wk.

**Milk and Blood Samples**

Milk samples were collected from the challenged quarter and the control quarter of each cow prior to challenge and at 2-h intervals during the first 8 h after infusion. Thereafter, samples were taken twice a day for 2 d and at 3, 5, 7, 14, and 21 d after the challenge. Bacterial counts in the milk were determined by preparing a 10-fold dilution series of milk in sterile saline and then plating 100 μl of sample on agar plates. Indirect mastitis indicators in the milk were also measured from all the milk samples. The NAGase activity was measured using the Milk NAGase Test Kit (Applied Diagnostics Corporation, Helsinki, Finland) (15). Trypsin inhibitor capacity (TIC) was determined using a commercial test kit (Milk Antitrpsin Test; Labsystems, Helsinki, Finland) as described earlier (15). Milk SCC were measured with a Fossomatic instrument (Foss Electric, Hillerød, Denmark) and very high SCC with a Coulter Counter particle counter (Coulter Electronics Ltd., Northwell, England) (9).

Milk endotoxin content was measured using a modified limulus amoebocyte lysate (LAL) test (E-tect; Farmos Diagnostica, Turku, Finland). All glassware that came into contact with the reagents was depyrogenized. Freeze-dried LAL was dissolved in pyrogen-free water. Thoroughly mixed milk samples were serially diluted in pyrogen-free water, and 50 μl of the LAL solution were mixed with an equal volume of diluted milk. The vials were incubated at 37°C in a water bath avoiding shaking. The results were read by gently inverting the vials. The test was deemed positive when the clot remained at the bottom of the vial. The endotoxin concentration was extrapolated from the lowest dilution that coagulated and multiplied by the sensitivity of the test. In this study, sensitivity was .18 to .25 EU (endotoxin units)/ml. A control series with endotoxin standards was always included in the test.

Twelve hours after the challenge, an aseptic blood sample was taken from the left jugular vein of each cow. The site of venipuncture was shaved and disinfected, and 10 ml of blood were transferred into an aerobic blood culture bottle (Hemobact™; Orion Diagnostica, Espoo, Finland). Blood cultures were incubated at 37°C and processed using standard methods. At the same time, a blood sample was taken to determine serum total calcium using standard techniques.

**Statistical Analyses**

Differences between treatments and challenge times were compared using repeated measures analysis of variance (22). A multivariate procedure was selected following significance established with Mauchly’s sphericity test. Logarithmic transformation was used to normalize the skewed distributions of data. Differences in general and local signs between groups were tested with crosstabulation and the Wilcoxon matched pairs, signed ranks test. Differences in serum calcium were tested with paired t tests. Milk endotoxin values were ranked into 12 groups and tested with the Wilcoxon test. Milk yields in different groups were also compared with the Wilcoxon test. Correlation between milk bacterial counts, endotoxin content, and the indicators of inflammation at different times were measured with Pearson’s correlation coefficients (22).

**RESULTS**

The response to challenge varied greatly among cows. In most cows, clinical signs were detectable 8 h after inoculation. For all the cows, clinical mastitis with moderate or severe systemic signs developed within 12 h. Mean serum calcium at 12 h was slightly under the physiological limit (mean, 2.16 mmol/L; range, 2.4 to 1.8; reference values, 2.2 to 2.6 mmol/L) (11). The values for the treatment groups did not differ significantly in this respect. No coliform bacteria were found in the blood samples.
taken 12 h postchallenge. Milk yield decreased to about a half of the prechallenge yield during the first 2 d but rapidly increased thereafter and was ~85% of the previous level at 3 wk for cows in all the groups.

All cows became infected. Bacterial counts in milk peaked 12 to 24 h after challenge, and milk endotoxin concentration peaked at 12 to 32 h. No significant differences existed between bacterial counts in milk for the different therapy groups (Figure 1). However, the number of bacteria in the milk decreased slightly faster in the group treated with TMP-S than in the other groups. Bacteria were eliminated from the challenged quarters of the TMP-S group within 5 d (median = 4) and from the colistin group within 7 d (median = 2.5). When antibiotics were not administered, bacteria were eliminated within 7 d (median = 5) in all but 1 cow; this cow still harbored low numbers

![Figure 1](image_url)
of bacteria at d 7 but no more 1 wk later. Milk endotoxin varied greatly among cows, and differences between groups were not statistically significant. In general, endotoxin was found in milk for as long as bacteria were cultured from the infected quarter. No endotoxin was found in the control quarters.

Data on milk NAGase and TIC for challenge times with antibiotic treatment versus no treatment are shown in Figures 2 and 3. Milk TIC peaked at 12 h, and NAGase activity and SCC peaked at 48 h. The increase was also evident in the control quarters. Mean milk SCC decreased and approached the preinfection value within 3 wk, and no differences were seen between the treatment groups. In the TMP-S group, antibiotic treatment seemed to enhance the decline in milk NAGase and TIC in the inoculated quarters, but the effect was not significant (Figures 2 and 3).

![Figure 2](image-url)

Figure 2. Mean milk NAGase in induced Escherichia coli mastitis in 6 cows after treatment with trimethoprim-sulfadiazine versus no treatment (top panel) and in 6 cows after treatment with intramammary colistin versus no treatment (bottom panel). Treatment with antimicrobials (●) or no treatment (○). Bars show standard errors. Initiation of the treatment is indicated by an arrow.
Correlations between the various indicators of inflammation in milk were highly significant at 2 and 3 wk postchallenge (Table 1). Correlation between bacterial count and endotoxin concentration in milk from 12 h to 32 h was significant and positive. These parameters also correlated positively \( (P < 0.05) \) with systemic and local signs at 24 h. The reduction in milk yield at that time was correlated with rises in body temperature and milk endotoxin content.

No correlation between indicators of inflammation and bacterial counts or endotoxin concentrations was established at the acute stage of infection. However, high numbers of bacteria and high endotoxin were significantly correlated with high SCC, NAGase, and TIC later (Table 1). Systemic signs disappeared

![Graph 1](image1)

**Figure 3.** Mean milk trypsin inhibitory capacity (TIC) in induced *Escherichia coli* mastitis in 6 cows after treatment with trimethoprim-sulfadiazine versus no treatment (left panel) and in 6 cows after treatment with intramammary colistin versus no treatment (right panel). Treatment with antimicrobials (■) or no treatment (○). Initiation of the treatment is indicated by an arrow.

TABLE 1. Pearson correlation coefficients between milk *Escherichia coli* counts, endotoxin content, NAGase activity, and trypsin inhibitory capacity (TIC) at different times following a challenge of 1.5 x 10^8 *E. coli* in two udder quarters of 12 lactating cows.

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*p < .05.
**p < .01.
***p < .001.

within 2 d in both groups. Local signs, such as swelling of the quarter and changes in milk appearance, were evident for somewhat longer than the systemic signs in all of the cows, but for no more than 1 wk, and for the same period in both groups. No differences were statistically significant between the two challenge times for any of the measured parameters.

Milk bacterial counts and endotoxin values were slightly higher at the first challenge as were NAGase, SCC, and TIC values from the 2 d after initiation of the experiment.

**DISCUSSION**

Therapeutic value of using antibiotics to treat coliform mastitis remains uncertain. The results from several trials have failed to show a beneficial effect of antibiotics compared with untreated controls or with administration of drugs without activity against coliforms in vitro (5, 10, 18, 19). Previous findings were supported by our study in which antimicrobials had no significant effect on experimentally induced coliform mastitis. Both antimicrobials used are considered to be drugs of choice for treating coliform mastitis (1), and the dosages used in this study were in accordance with those recommended in the literature (16). The bacterial strain used in this experiment was highly sensitive in vitro to the antimicrobials used, and drug concentrations above MIC values were maintained in milk (16, 25).

Response to challenge varied greatly among individual cows. The possible effect of repeated challenge was eliminated by the trial design. In this model, the same cow was the experimental and the control unit, which eliminated the confounding effect of using different cows with different responses. Bacterial counts in milk continued increase at 8 h when the first clinical signs could be detected. These results contrast with those of Hill et al. (7).

Milk bacterial counts and endotoxin were, as expected, significantly correlated during the acute phase of the disease. Also, correlation was positive between high values of these parameters and severe clinical signs. Lohuis et al. (13) showed that severe signs of experimental *E. coli* mastitis are associated with greater long-term reductions in milk yields; here, this connection was only seen at the acute stage. No correlation existed among the various milk parameters at the acute stage because these parameters reflect different phenomena that do not occur simultaneously.
Correlation is positive between numbers of bacteria and the release of endotoxin (21), which results in increasing destruction of the udder. This effect was seen in our study, in which high numbers of bacteria and high endotoxin concentrations preceded a slower decline in indicators of inflammation in the milk (Table 1). In particular, high endotoxin values were followed by high milk NAGase values, which in turn reflected tissue damage. Milk endotoxin and status of the cow have previously been compared in one field study in which high milk endotoxin concentration predicted a poor prognosis (12). High milk endotoxin concentrations generated by Gram-negative bacteria have also occurred in gangrenous mastitis (6). High doses of the combination TMP-S may be bactericidal, at least during the first hours after drug administration. Release of large amounts of bacterial endotoxin, as has occurred after treatment with some bactericidal drugs (20, 21), was not observed in this study. The TMP-S treatment slightly hastened elimination of bacteria, and the inflammatory reaction in the quarters declined more rapidly than that of the control (Figures 1 and 2). However, the effects were not statistically significant.

The assumption that endotoxin does not generally reach the circulation was supported in our study by the finding that no endotoxin was found in the control quarters. Colistin inactivates endotoxin in mastitic milk in vitro (23). In the present study, milk endotoxin in the group treated with colistin did not differ from that in the untreated control group. Intramammary administration of antibiotics can be contraindicated in coliform mastitis because phagocytosis in the udder may be disturbed (4, 24). Colistin was not included in the in vitro studies on phagocytosis referred to, but polymyxin E had no significant negative effects.

Using this experimental model, antibacterial treatment is not likely to be of value for treating coliform mastitis during the middle or late lactation period. The long withdrawal times of antibiotics used in this study make this type of therapy very costly. Sometimes this treatment has been recommended based on the risk of bacteremia developing in cows infected with coliforms (4), but bacteremia did not develop in any of the cows included in this study. A related issue is how to diagnose the disease sufficiently early to avoid the need for antibi-otic therapy. For this purpose, rapid cow-side tests to detect milk endotoxin could be useful.

In puerperal cows, antimicrobial therapy remains necessary because host defense mechanisms may be compromised and elimination of bacteria is not efficient (8). However, the most important part of therapy in coliform mastitis undoubtedly should be directed against the detrimental effects of the endotoxin-mediated inflammatory reaction and consist of anti-inflammatory agents (2), fluid therapy, and frequent milking.

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REFERENCES

TREATMENT OF ESCHERICHIA COLI MASTITIS
