FULL PAPER *Theriogenology*

Therapeutic Efficacy of Mammary Irrigation Regimen in Dairy Cattle Diagnosed with Acute Coliform Mastitis

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ABSTRACT. The objective of this field study was to determine the therapeutic efficacy of mammary irrigation for the treatment of dairy cattle diagnosed with acute coliform mastitis caused by gram-negative bacteria. Additionally, the effects of different mammary irrigation regimen fluids such as ozone water and normal saline were compared. Dairy cattle clinically diagnosed with acute coliform mastitis (n=57) were enrolled in the study, randomly assigned to 1 of 3 groups, and received the following treatments: systemic antibiotic administration (SAA group; n=40), mammary irrigation regimen (MIR group; n=10), and both treatments (MIX group; n=7). Significant antipyretic effects, as assessed by rectal temperature measurement, were observed in the MIX and MIR groups. Although 2 irrigating fluids were used, namely, ozone water and normal saline, no significant difference was observed between the 2 groups. Fourteen days after the onset of the treatments, the milk yield recovery rate in MIR group tended to be higher (p=0.06) than that in the SAA group. Additionally, after 30 days of treatment, the MIR group cows demonstrated significantly higher successful recovery rates ($p<0.05$) than the SAA group cows. These results indicate that mammary irrigation with normal saline is an effective treatment for acute coliform mastitis in dairy cattle.

KEY WORDS: antibiotic, coliform mastitis, mammary irrigation, ozone water, therapy.

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Acute coliform mastitis, caused by gram-negative bacteria such as *Escherichia coli* (many serotypes), *Klebsiella* spp. (numerous capsular types), and *Enterobacter aerogenes*, is one of the major sources of economic loss in dairy farms because its effects tend to be fatal [2]. In such cases, the milk yield of the infected cows decreases sharply, and occasionally, complete loss of a mammary quarter occurs. The infected cows are often culled because of low milk yield, thereby causing higher economic damage to dairy farmers. Therefore, an effective treatment that minimizes the adverse impact of coliform mastitis on milk yield is urgently required. Endotoxic shock resembles septic shock and develops in the infected cows due to severe inflammatory response to endotoxins which are present in the outer cell wall of gram-negative bacteria. Recent medical researches, including several *in vitro* and *in vivo* studies, have demonstrated the antibiotic-induced release of endotoxins by gram-negative bacteria on exposure to antibiotics [7, 12, 13]. Similarly, in the case of cows undergoing treatment for clinical coliform mastitis caused by gram-negative bacteria, there are reports on the possibility that antibiotic therapy can increase morbidity and mortality, which may be attributed to the release of endotoxins [4]. Therefore, although systemic antibacterial therapy may favorably influence the outcome of this disease in many cases [8, 19], inappropriate choice of antibiotics may increase mortality due to the release of a large amount of endotoxins [14, 20].

The clinical benefits of mammary irrigation and bacteriostatic antibiotics therapy administered by intravenous injection for coliform mastitis had been documented [5]. However, our previous study demonstrated that the amounts of induced-endotoxin induced by bacteriostatic antibiotics were not different from those by bactericidal antibiotics [18]. Moreover, in farm animal practices, the impact of antibiotic use has recently been considered to be important not only for preventing the emergence of drug resistance but also from the viewpoint of food safety and public health [16]. On the basis of these findings, we speculated that mammary irrigation therapy, rather than systemic antibacterial therapy, is useful for the treatment of acute coliform mastitis in cattle because it (1) eliminates both microorganisms and endotoxins and (2) disrupts endotoxin production from the infected mammary glands. Recently, we conducted *in vitro* experiments, and demonstrated for the first time that the volumes of endotoxins released from *E. coli* after sterilization by ozone exposure were lower than those released subsequent to antibiotic treatments [18].

Therefore, in this field study, we investigated the therapeutic efficacy of the mammary irrigation regimen (MIR) in dairy cattle clinically diagnosed with acute coliform mastitis caused by gram-negative bacteria. Further, we compared its efficacy with that of systemic antibiotic therapy by using the following clinical parameters: (1) recovery from clinical symptoms, (2) recovery of milk yield, and (3) incidence of case-related death and culling due to decreased lactation. Additionally, the efficacy of ozone water as an irrigation

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medium was also compared with that of sterile normal saline, with the same clinical parameters.

MATERIALS AND METHODS

Selection of cows for treatment: Between 2005 and 2007, lactating cows diagnosed with acute coliform mastitis were selected from 30 farms and enrolled in this study. The diagnosis was established in the first examination which was conducted as previously reported [15]. The diagnosis was based on the presence of typical clinical symptoms of the mammary gland (hard and swollen quarter), accompanied by at least 2 of the following general clinical symptoms that are suggestive of systemic involvement: (1) rectal temperature > 40°C, (2) watery milk, (3) anorexia, (4) decreased milk yield, or (5) shock (increased heart rate, tenting of eyelids, shivering, and slow capillary refilling time). None of the cows had a clinical history of chronic mastitis or other complications. Simultaneously with the above clinical examinations, milk samples were collected from the diagnosed cows and used for bacterial examination. Isolation of the bacteria in milk samples was performed by incubation in the CHROMagar™ Orientation (Kanto Chemical Co., Inc., Tokyo, Japan) under aerobic conditions at 37°C for 24 hr. The isolated bacteria were identified on the basis of the criteria detailed in a previous report [6]. In the present study, a total of 57 cows were enrolled, and gram-negative bacteria were eventually isolated from the milk samples obtained from all the cows.

Treatment administration and procedures: Cows that fulfilled the enrollment criteria underwent 1 of the following 2 treatments: (1) systemic antibiotic administration, SAA (n=47) or (2) MIR (n=10). The cows were randomly administered 1 of the above 2 first-line treatments. Immediately after detection of the infection, the SAA cows were systemically administered a suitable antibiotic (e.g., cefazolin (5 mg/kg), aminobenzyl penicillin (8 mg/kg), kanamycin (10 mg/kg), and enrofloxacin (5 mg/kg). In contrast, the cows in the MIR group received only mammary irrigation treatment and did not receive systemic antibiotics. Following the first treatment, some cows in the SAA group were randomly assigned to undergo additional MIR treatment (MIX group; n=7) in order to assess the synergistic effects of the 2 treatments (Fig. 1). In all cases, the cows of the MIX group were administered MIR immediately after SAA. One session of MIR (in the MIX and MIR groups) involved flushing one of the following solutions (1–3 *l*) several times into the mammary gland: (1) ozone water (n=7) or (2) normal saline (n=10). The effects of the 2 solutions on the recovery process were compared. The contents of the MIR solution were ozone water (n=3) and normal saline (n=7). In addition to these specific treatments for mastitis, all cows in the present study were administered general supportive therapies, including intravenous fluids, and anti-inflammatory agents and other drugs.

Preparation of ozone water: An ozone-generating equipment (OZONE DASH TWIN 30 system; Toyota Shatai Co., Aichi, Japan) was used to produce ozone water. This equipment produces ozone via the electric discharge method and injects ozone into water (0.8 mg/*l*). In the food industry, the concentration 0.8 mg/*l* is widely accepted as an effective and safe concentration. Due to the unstable nature of ozone water, it was used for the treatment immediately after it was generated.

Clinical observations after individual systemic treatment: Clinical symptoms such as rectal temperature, milk color (watery or not), anorexia, milk yield, and shock were recorded both before the systemic treatments (day 0) and on the day after the treatments (day 1). The conditions of milk yield recovery were observed 14 days after the clinical diagnosis. Recovery was classified as follows: (1) perfect recovery without local and systemic clinical signs, (2) failure of lactation in 1 quarter without systemic signs, and (3) failure of lactation in all quarters. Additionally, on the basis of the data files from the Total Clinical Recording System at the Yamagata Veterinary Clinical Center, the efficacy of each systemic treatment was evaluated using categorical outcome variables, including the incidence of treatment failures (culling or death) or survival within 30 days after the clinical diagnosis.

Statistical analysis: Fisher's exact test was used to compare the treatment efficacy of SAA and MIR. The McNemar test was used for comparison of the changes in the clinical symptoms between days 0 and 1. The Kruskall-Wallis test was used to compare milk yield after various treatments and irrigation with different irrigation fluids. The paired *t* test was used to compare changes in the rectal temperature due to various treatments. Treatment effects were considered statistically significant at $p<0.05$ and

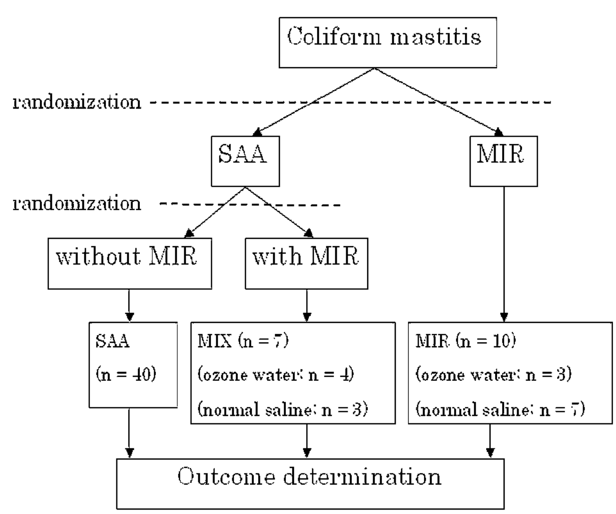

Fig. 1. Scheme of the treatment protocol used for severe clinical mastitis in the present study. Cows were randomly assigned to 2 groups, and received either systemic antibiotic administration (SAA) or mammary irrigation regimen (MIR). Some cows of the SAA group were randomly administered additional mammary irrigation treatment regimen (MIX).

tended to be significant at 0.05<p<0.10.

RESULTS

A total of 57 cows were enrolled in this study. Figure 2 shows the rectal temperatures of the SAA, MIX, and MIR group cows on days 0 and 1. In the MIX and MIR groups, the rectal temperatures decreased significantly after 24 hr of the treatment from 40.6 ± 0.9°C to 38.7 ± 0.3°C (p<0.01) and from 40.1 ± 1.1°C to 39.2 ± 0.6°C (p<0.05), respectively. No significant difference was observed in the SAA group. In the MIR group, no significant difference was observed with regard to the rectal temperature between days 0 and 1 (ozone water (n=3): 40.4 ± 1.4°C to 39.0 ± 0.6°C and normal saline (n=7): 40.0 ± 1.1°C to 39.2 ± 0.7°C).

Figure 3 shows the changes in the clinical symptoms after each treatment in the SAA, MIX, and MIR group cows on days 0 and 1. As shown in Fig. 3-A and Fig. 3-D, in the SAA group, between days 0 and 1, the incidence of watery milk significantly increased after treatment (p<0.05), and that of shock tended to increase (p<0.10). In the MIX and MIR groups, no significant differences were observed between days 0 and 1 with regard to any parameter.

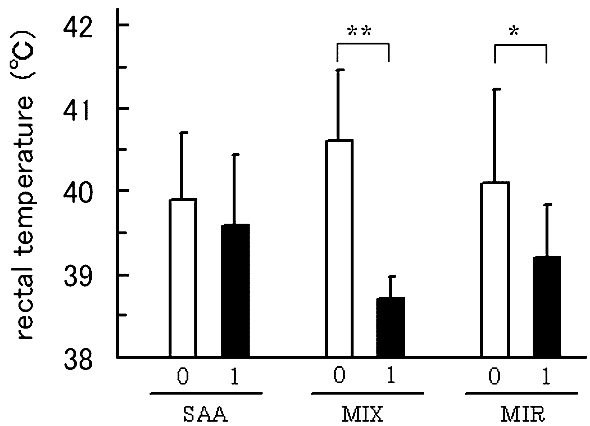

Fig. 2. Rectal temperatures after systemic antibiotic administration (SAA; n=40), mammary irrigation regimen (MIR; n=10), and both treatments (MIX; n=7) on days 0 (white bar) and 1 (black bar) are shown. Data are expressed as mean ± SD. **: significant difference at p<0.01; *: significant difference at p<0.05.

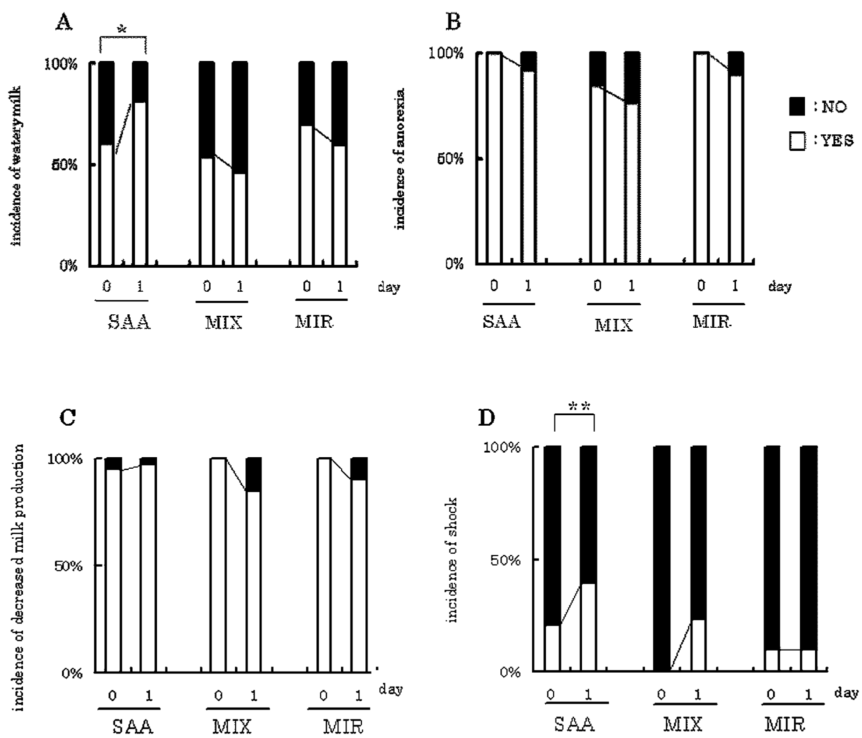

Fig. 3. Clinical symptoms after systemic antibiotic administration (SAA; n=40), mammary irrigation regimen (MIR; n=10), and both treatments (MIX; n=7) on days 0 and 1 (the day after the treatment). (A): Incidence of watery milk. (B): Incidence of anorexia. (C): Incidence of decreased milk yield. (D): Incidence of shock. The white bar indicates YES, and the black bar indicates NO. *: significant difference at p<0.05; **: significant difference at p<0.10.

Table 1. Milk production recovery according to treatment group and *p* value between each treatment

Treatment group*	Milk yield recovery**			*p* value vs.	
	I	II	III	MIX	MIR
SAA (n=20)	3 (15.0%)	8 (40.0%)	9 (45.0%)	0.79	0.06
MIX (n=4)	1 (25.0%)	2 (50.0%)	1 (25.0%)	–	0.6
MIR (n=8)	5 (62.5%)	2 (25.0%)	1 (12.5%)	–	–
Total (n=32)	9	12	11		

*: SAA: systemic antibiotic administration, MIX: systemic antibiotic administration with the mammary irrigation regimen, MIR: mammary irrigation regimen, and **: classification of recovery. I, perfect recovery without local and systemic signs; II, failure of lactation in a quater without any systemic signs; and III, lactation failure in all quarters.

Table 1 shows the recovery of milk yield after each treatment. Of the 57 cases, 32 could be evaluated for the recovery of milk yield at 14 days after treatment onset. The recovery rates of milk yield in the MIR group tended to be higher (p=0.06) than those in the SAA group. The percentages of complete recovery without local and systemic symptoms in the SAA, MIX, and MIR groups were 15.0% (3/20), 25.0% (1/4), and 62.5% (5/8), respectively. Among the 8 cases in the MIR group, in all the 5 cases of complete recovery without local and systemic symptoms, normal saline was used as the irrigation fluid.

Table 2 shows the clinical outcomes of the SAA, MIX, and MIR group cows at 30 days after treatment. The success rates, assessed on the basis of the clinical symptoms, in the MIX (71.4%; 5/7) and MIR (80.0%; 8/10) groups were significantly higher ($p<0.05$) than those in the SAA group (37.5%; 15/40). No significant difference was observed between the MIX and MIR groups. Among the 10 cows in the MIR group, the success rates in the case of irrigation with ozone water and normal saline were 66.7% (2/3) and 85.7% (6/7), respectively.

Table 2. Clinical outcome according to treatment group and relative risk

Treatment group*	Clinical Outcome Therapy success
SAA (n=40)	15 (37.5%)$^{a)}$
MIX (n=7)	5 (71.4%)$^{b)}$
MIR (n=10)	8 (80.0%)$^{b)}$
Total (n=57)	28 (49.1%)

*: SAA: systemic antibiotic administration, MIX: systemic antibiotic administration with the mammary irrigation regimen, and MIR: mammary irrigation regimen. a,b: Values in the same row with different superscripts statistically differ ($p<0.05$).

DISCUSSION

The therapeutic effect of MIR in dairy cattle diagnosed with acute coliform mastitis caused by gram-negative bacteria was investigated. Our results clearly demonstrated that cows receiving MIR had higher cure rates than those receiving SAA alone. These results show that MIR is more effective than suggested by previous studies, which reported the beneficial effects of systemic antibacterial therapy in cows afflicted with coliform mastitis [8, 19].

In the present study, greater aggravation of clinical symptoms, such as watery milk and shock, was observed in the SAA group on day 1. Several studies on pathogenicity toward mammary tissues have reported that the adherence of *E. coli* and *K. pneumoniae* to epithelial tissue might not play a major role in the pathogenesis of bovine mastitis [3, 11]. SAA may result in increased antibiotic-induced endotoxin release in the mammary gland and accelerate the immune response, resulting in prolonged inflammation. Therefore, the prevention of mammary tissue injury caused by inflammation may be achievable through the use of MIR alone. Accordingly, the clinical benefits of SAA as a first-line treatment regimen for clinical coliform mastitis are questionable.

In this study, we used ozone water, which has anti-microbial activity, as a mammary irrigation fluid. It is well recognized that ozone has sterilization capabilities and is applied in various fields [17]. It is being increasingly used as a treatment tool in mastitis [9], especially for the treatment of coliform mastitis, where intramammary application of ozone gas is used due to its high cure rate. It was reported that treatment with ozone released fewer endotoxins in the milk than treatment with antibiotics; further, no iatrogenic endotoxic shock occurred as an adverse effect, unlike that in the case of antibiotic treatment [10]. In addition, it has been reported that ozone may result in the downregulation of the cytotoxic activity of immunocytes [1, 21, 22]. Therefore, we speculated that using ozone water as an irrigation fluid during MIR as the first line of treatment for coliform mastitis was possible to reduce mortality. In the present study, the use of different irrigation fluids (ozone vs. normal saline) produced no significant differences on the milk yield recovery and cure rates. These results, for the first time, indicated that the physical elimination of both microorganisms and endotoxins from the mammary glands by flushing might be essentially important, rather than the sterilization effect of irrigation fluids. Although we recently confirmed using *in vitro* evaluation that ozone exposure decreases the amount of endotoxins released from *E. coli* [18], we could not confirm the efficacy of ozone water in the *in vivo* evaluation in the present field study.

Results from the present field study are clearly indicative of the effects of MIR on the mortality and morbidity of severe clinical mastitis caused by gram-negative bacteria. In conclusion, mammary irrigation with normal saline was effective for acute coliform mastitis in dairy cattle, indicating the first-choice treatment in severe clinical mastitis in field practice.

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