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Acute *Escherichia coli* Mastitis in Dairy Cattle: Diagnostic Parameters Associated with Poor Prognosis

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ABSTRACT. This study aimed to identify the diagnostic characteristics associated with poor prognosis and mortality in dairy cows with acute clinical *Escherichia coli* mastitis. On 17 dairy farms, 24 dairy cows with acute *E. coli* mastitis that had received therapeutic treatment were categorized into 2 groups by outcome: 17 cows that recovered (survivors) and 7 cows that died or were euthanized (non-survivors). Two days after onset of acute *E. coli* mastitis, dysstasia was observed in non-survivors, but not in survivors. Compared with survivors, significantly increased hematocrit (HCT) values and non-esterified fatty acid (NEFA) concentrations, and significantly decreased antithrombin activity and platelet counts were found in non-survivors on days 2 and 3 after therapy. Dysstasia, associated with decreased antithrombin activity and platelet counts, and with increased HCT and NEFA concentrations, was considered to be the major prognostic indicator associated with high mortality after therapeutic treatment in acute *E. coli* mastitis.
KEY WORDS: bovine, diagnostic parameter, *Escherichia coli* mastitis, fatal outcome

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Acute coliform mastitis is a common and usually fatal disease in lactating dairy cows. Endotoxemia and disseminated intravascular coagulation (DIC) in cows with acute *Escherichia coli* mastitis are generally recognized as the causes of fatality. Bacteremia has been reported to occur in 32% [3, 23] to 75% [11] of cows with naturally occurring coliform mastitis. Endotoxemia [11], metabolic acidosis [3], uremia [2] and increased aspartate aminotransferase (AST) activity [3] are commonly observed in cows with naturally occurring coliform mastitis. Many studies have used lipopolysaccharides (LPSs) [21] to reproduce the clinical signs of mastitis in dairy cows, but fatalities were not seen [16, 18, 20]. Other studies have experimentally infected bovine mammary glands with *E. coli*, inducing temporary pyrexia accompanied by cytokine increases; however, infected cows appeared able to eliminate the bacteria from the infected gland without therapeutic intervention [8, 13, 17].

Diagnostic parameters are useful to predict the fatality and poor prognosis due to acute *E. coli* mastitis. The objective of this study was to identify the diagnostic parameters that could be used in clinical practice to predict poor prognosis or mortality in cows with naturally occurring acute *E. coli* mastitis.

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MATERIALS AND METHODS

Dairy cows: Holstein: Holstein dairy cows with naturally occurring acute *E. coli* mastitis, diagnosed and treated by local veterinarians, were monitored. All cows belonged to one of 17 local dairy herds in the Kitami district, Hokkaido, Japan. Clinical signs in cows with *E. coli* mastitis, such as dysstasia, diarrhea and cool extremities, were recorded by veterinarians and dairy farmers throughout the observation period. The 24 dairy cows used for this study had confirmed *E. coli* infection (as isolated from infected quarter milk) and one or more of the following findings on day 1: rectal temperature greater than 40°C, heart rate greater than 120 beats/min, respiratory frequency greater than 30 breaths/min and blood total leukocyte count less than 5,000/μl.

The 24 affected cows comprised 17 cows that survived (survivors) and 7 cows that died or were euthanized (non-survivors) after therapeutic treatment. Among the non-survivors, 1 cow died on day 3, another died on day 4 and 5 cows were euthanized (1 per day) on days 4, 5, 6, 7 and 8 after the onset of acute *E. coli* mastitis. In this study, because cows were euthanized only when therapeutic treatment had failed to improve their condition, euthanasia was considered to be an equivalent outcome to natural death. Survivors comprised 17 cows in which clinical symptoms resolved on days 3, 4, 5, 6 and 8.

Therapy: All cows were administered kanamycin sulfate (4,000–6,000 mg/cow/day) intramuscularly, 7.2% sodium chloride solution (2,000 ml/cow/day) intravenously and a combination of kanamycin sulfate (300 mg/cow/day) and penicillin-G-procaine (300,000 U/cow/day) as an intramammary infusion. Additionally, 1,000 U of heparin sodium

Table 1. Comparison of age, parity, days after parturition, milk yield and somatic cell counts in cows with acute *Escherichia coli* mastitis between non-survivors and survivors

	Non-survivors		Survivors	
	n	Prior to onset	n	Prior to onset
Age	7	7.2 ± 1.7*	17	4.9 ± 1.7
Parity	7	4.7 ± 1.5*	13	2.9 ± 1.4
Days after parturition	7	138.1 ± 98.2	17	128.4 ± 91.1
Milk yield (*l*/day)	6	35.1 ± 9.9	13	34.1 ± 5.0
Somatic cell count ($\times 10^4$/m*l*)	6	14.5 ± 18.2	13	26.6 ± 44.0

Data were expressed as mean ± SD. n: Number of cows. Non-survivors comprised died or euthanized cows, and survivors were defined as survived or recovered cows from acute *E. coli* mastitis. *Significantly different from that of survivors (P<0.05).

Table 2. Comparison of clinical symptoms of cows with acute *Escherichia coli* mastitis between non-survivors and survivors

	Days	Non-survivors	Survivors
Clinical symptom	after onset	%[a] (N1 / N2)	%[a] (N1 / N2)
Dysstasia	1	71 (5/7)*	0 (0/17)
	2	100 (7/7)*	0 (0/17)
	3	83 (5/6)*	0 (0/12)
Cool extremity	1	86 (6/7)	59 (10/17)
	2	71 (5/7)	41 (7/17)
	3	17 (1/6)	0 (0/12)
Diarrhea	1	29 (2/7)	18 (3/17)
	2	29 (2/7)	24 (4/17)
	3	0 (0/6)	8 (1/12)

Non-survivors comprised died or euthanized cows, and survivors were defined as survived or recovered cows from acute *E. coli* mastitis. a) The proportion (%) was calculated as the number of cows showing each clinical symptom (N1)/ the number of observed cows (N2). *Significantly different from that of survivors (P<0.05).

Table 3. Comparison of rectal temperature, heart rate and respiratory frequency of cows with acute *Escherichia coli* mastitis between non-survivors and survivors

	Days	Non-survivors		Survivors	
Clinical signs	after onset	n		n	
Rectal temperature (°C)	1	7	39.4 ± 0.4*	17	39.9 ± 0.9
	2	7	38.7 ± 0.7	16	39.1 ± 0.6[c]
	3	6	39.0 ± 0.6	12	39.0 ± 0.5[c]
Heart rate (beat/min)	1	7	109.1 ± 11.0	17	104.9 ± 17.7
	2	6	94.7 ± 19.2	16	96.9 ± 10.0
	3	5	93.6 ± 15.1	12	99.2 ± 13.4
Respiration frequency (breath/min)	1	7	49.7 ± 10.0	17	44.9 ± 12.8
	2	7	44.3 ± 16.2	15	43.2 ± 12.4
	3	6	50.0 ± 15.1	12	42.9 ± 12.2
Proportion of cows showing 2 or more clinical signs [a]	1	7	86[a] (6/7)[b]	17	94[a] (16/17)[b]
	2	7	86[a] (6/7)[b]	17	71[a] (12/17)[b]
	3	6	83[a] (5/6)[b]	12	42[a] (5/12)[b]

Data were expressed as mean ± SD. n: Number of cows. Non-survivors comprised died or euthanized cows, and survivors were defined as survived or recovered cows from acute *E. coli* mastitis. a) The proportion (%) was calculated as the number of cows showing 2 or more of the following signs: rectal temperature >40°C, heart rate >120 beats/min, respiratory frequency >30 breaths/min and white blood cell counts <5,000 /μl. b) The values in the parentheses indicate the number of cows showing clinical signs / the number of observed cows. c) Values were significantly different from that of day 1 by survivors or non-survivors (P<0.05). *Significantly different from that of survivors (P<0.05).

(25–50 m*l*/cow/day), physiological saline solution (2,000–8,000 m*l*/cow/day) and 5% glucose (2,000–5,000 m*l*/cow/day) were intravenously administered when judged necessary. Frequency and dosage of medical treatments were identical for both survivors and non-survivors.

Milk and blood sample collection: Quarter milk [9] and peripheral blood from affected cows were collected according to the standard procedures during the first 3 days after the onset of clinical symptoms. Quarter milk from affected cows was collected aseptically into sterilized culture tubes and was sent to a diagnostic laboratory (Kishimoto Medical Lab., Tomakomai, Japan) for identification of isolates. *E. coli* isolates were identified by a specific biochemical system (Sysmex-bioMerieux Co., Ltd., Tokyo, Japan) using a specific kit (VITEK 2; Kishimoto Medical Lab.).

Analyses for 19 blood serum biochemicals were conducted during the first 3 days after the onset of acute symptoms. Peripheral blood was collected from the jugular vein into evacuated tubes (BD Vacutainer SST II K2 EDTA; Becton, Dickinson and Co., Tokyo, Japan) and into tubes containing 3.8% sodium citrate as an anticoagulant (NP-CS0457; Nipro, Tokyo, Japan).

Samples were not obtained for one non-survivor, which died on day 3 before samples could be collected, and for 5 survivors, from which samples on day 3 were not collected.

Blood biochemical analysis: Serum from affected cows

Table 4. Comparison of selected measures of cows with acute *Escherichia coli* mastitis between non-survivors and survivors

Selected measures	Days after onset	Non-survivors		Survivors	
		n		n	
Hematocrit (%)	1	7	32.7 ± 5.8	17	29.6 ± 2.8
	2	7	33.5 ± 5.7*	17	27.9 ± 3.3
	3	6	34.8 ± 6.6*	12	27.5 ± 3.3
Red blood cell count ($\times10^4$/m*l*)	1	7	686.9 ± 110.6	17	637.8 ± 60.7
	2	7	697.6 ± 100.8*	17	604.4 ± 78.9
	3	6	717.8 ± 117.1*	12	587.5 ± 71.5
White blood cell count ($\times10^3/\mu l$)	1	7	1.7 ± 1.9	17	2.0 ± 1.3
	2	7	2.7 ± 3.5	17	3.5 ± 3.3
	3	6	6.7 ± 10.5	12	5.6 ± 3.6[c)]
Platelet count ($\times10^4$/m*l*)	1	7	15.1 ± 5.9	17	18.6 ± 9.1
	2	7	13.2 ± 2.6*	17	20.4 ± 9.9
	3	6	9.8 ± 3.3*	12	19.9 ± 10.1
Aspartate aminotransferase activity (IU/*l*)	1	7	123.3 ± 40.4	17	106.9 ± 52.9
	2	7	259.6 ± 189.2	17	109.4 ± 77.6
	3	6	504.8 ± 352.4[*a)]	12	107.3 ± 52.5
Blood urea nitrogen concentration (mg/d*l*)	1	7	18.9 ± 3.7	17	18.1 ± 5.6
	2	7	17.1 ± 3.0	17	15.6 ± 3.5
	3	6	16.4 ± 3.3*	12	11.6 ± 2.8[c)]
Non-esterified fatty acid concentration (mEq/*l*)	1	7	0.6 ± 0.4	17	0.4 ± 0.2
	2	7	0.7 ± 0.2*	16	0.4 ± 0.2
	3	6	0.9 ± 0.2*	12	0.4 ± 0.2
Fibrinogen concentration (mg/d*l*)	1	7	264.0 ± 43.9	17	280.6 ± 67.1
	2	7	293.1 ± 30.6	17	319.7 ± 44.9
	3	6	343.2 ± 43.4[a)]	12	353.2 ± 55.0[a)]
Antithrombin activity (%)	1	7	142.0 ± 22.4	17	154.5 ± 21.1
	2	7	119.9 ± 18.9*	17	142.4 ± 23.0
	3	6	100.0 ± 11.9[*a)]	12	134.6 ± 23.6
Prothrombin time (s)	1	7	35.8 ± 9.4	17	34.0 ± 7.6
	2	7	53.8 ± 17.5	17	40.2 ± 8.0
	3	6	57.8 ± 16.3[*a)]	12	34.9 ± 7.9

Data were expressed as mean ± SD. n: Number of cows. Non-survivors comprised died or euthanized cows, and survivors were defined as survived or recovered cows from acute *E. coli* mastitis. a) Values were significantly different from that of day 1 by survivors or non-survivors ($P<0.05$). *Significantly different from that of survivors ($P<0.05$).

was collected to determine AST activity and concentrations of blood urea nitrogen (BUN), calcium, chlorine, potassium, sodium, non-esterified fatty acid (NEFA), phosphorus and total cholesterol. Plasma samples with EDTA were used for determining hematocrit (HCT), platelet count, red blood cell count and white blood cell count. Plasma samples with sodium citrate anticoagulant were used for determining activated partial thromboplastin time, antithrombin activity, fibrinogen concentration and prothrombin time.

Activated partial thromboplastin time and prothrombin time were measured with an automated clot detector instrument (CA-6000 Blood Analyzer; Sysmex, Kobe, Japan) with fibrin formation as the endpoint. Fibrinogen concentration was extrapolated from results of the thrombin time test. Antithrombin activity was measured by a chromogenic assay (N-Assay L AT III; Nittobo, Tokyo, Japan) with an automated instrument (JCA-BM12; JEOL, Tokyo, Japan). Serum AST, BUN, calcium, NEFA, phosphorus and total cholesterol concentrations were measured with an automated analyzer (Kishimoto Medical Lab.). Chlorine, potassium and sodium concentrations were measured by ion-selective electrode methods. HCT, platelet count, red blood cell count and white blood cell count were measured with an automated instrument (Analyzer SE-9000; Sysmex).

Statistical analysis: All statistical analyses were performed using SPSS 17.0 software (SPSS Japan, Tokyo, Japan). Differences between the 2 groups were analyzed by the 2-sample *t*-test or the 2-sample *t*-test with Welch's correction. A chi-squared test was performed to make a comparison between the 2 groups in terms of proportion of cows with clinical symptoms (%). Differences between days (day 1 vs. days 2 and 3) were analyzed by repeated measure analysis of variance with post hoc Tukey's test where appropriate. Correlation coefficients among measured values were obtained by Pearson's correlation. A *P*-value of less than 0.05 was considered significant.

Table 5. Correlation coefficients of antithrombin activity, platelet counts and blood urea nitrogen concentration with other measures in cows with acute *Escherichia coli* mastitis on days 2 and 3 after the onset of mastitis

(Days after onset) Selected measures	Non-survivors	Survivors
	Antithrombin activity (%)	
(Day 2)		
Prothrombin time (s)	–0.14	–0.52*
Non-esterified fatty acid (mEq/*l*)	–0.53	–0.63*
(Day 3)		
Prothrombin time (s)	–0.30	–0.75*
Non-esterified fatty acid (mEq/*l*)	0.49	–0.37
	Platelet count ($\times 10^4$/m*l*)	
(Day 2)		
Prothrombin time (s)	–0.48	–0.48
(Day 3)		
Prothrombin time (s)	–0.59	–0.64*
	Blood urea nitrogen concentration (mg/d*l*)	
(Day 2)		
Hematocrit (%)	0.43	0.43
Red blood cell count ($\times 10^4$/m*l*)	0.38	0.58*
(Day 3)		
Hematocrit (%)	0.81*	0.75*
Red blood cell count ($\times 10^4$/m*l*)	0.79	0.65*

Non-survivors comprised died or euthanized cows, and survivors were defined as survived or recovered cows from acute *E. coli* mastitis. *Significance at $P<0.05$.

RESULTS

Compared with survivors, non-survivors had higher age and higher parity ($P<0.05$) (Table 1). Dysstasia in mastitic cows occurred in all non-survivors on day 2, but was not observed in survivors ($P<0.01$, Table 2). Cool extremities were observed more frequently in non-survivors than in survivors. Diarrhea was observed in both non-survivors (28.6%, 2/7) and survivors (23.5%, 4/17). No significant differences in rectal temperature, heart rate, respiratory frequency or white blood cell counts were observed between non-survivors and survivors in the first 3 days (Tables 3 and 4).

Non-survivors showed significantly higher HCT, red blood cell counts and NEFA concentrations ($P<0.05$) than survivors on days 2 and 3, but these values were not significantly different on day 1 (Table 4). Non-survivors showed significant decreases in antithrombin activity ($P<0.05$) and platelet counts, and showed prolonged prothrombin time ($P<0.05$) (Table 4). A significant increase in fibrinogen concentration was equally observed in both groups ($P<0.01$). Activated partial thromboplastin time and concentrations of calcium, chlorine, potassium, sodium, phosphorus and total cholesterol on days 1, 2 and 3 did not significantly differ between the groups (data not shown). Although antithrombin activity was not correlated with prothrombin time and NEFA concentration in non-survivors (Table 5), it was negatively correlated with prothrombin time and NEFA concentration in survivors ($P<0.05$). Similarly, in non-survivors, platelet count was not correlated with prothrombin time, but it was negatively correlated with prothrombin time in survivors on day 3 ($P<0.05$). BUN concentration was positively correlated with HCT and red blood cell count in survivors ($P<0.05$; Table 5).

DISCUSSION

Because pyrexia, tachycardia, panting and leukopenia were equally observed in both survivors and non-survivors, they could not be applied to predict poor prognosis or mortality of dairy cows with acute *E. coli* mastitis. In addition, the proportion of cows showing 2 or more of these clinical signs after therapeutic treatment did not differ between the 2 groups.

Older cows with *E. coli* mastitis were found to be at higher risk for the fatal outcomes than younger cows. Our study confirmed that higher mortality of cows with *E. coli* mastitis was associated with higher age and higher parity [22], which may be related to decreased neutrophil function [6] and higher frequency of fatty liver [7] in older cows, as suggested previously [15]. In our blood biochemical findings of cows with *E. coli* mastitis, significantly higher serum NEFA concentrations were found in non-survivors, indicating that increased serum NEFA may be caused by the mobilization of fat from adipose tissue to the liver [10, 12]. This excess mobilized NEFA, which accumulates as triacylglycerol in the liver, results in fatty liver or ketosis and thereby impairs metabolic liver function [10, 12]. Healthy cows can clear LPS from the blood stream within 30 min after LPS intravenous administration. However, cows with fatty liver cannot clear LPS by 6 hr after administration [1]. Therefore,

in severe *E. coli* mastitis, impaired hepatic functions of dairy cows with increased NEFA could lead to difficulty in LPS detoxification and to decreased antithrombin synthesis, putting them at high risk of death.

The increased HCT and red blood cell counts in non-survivors were clearly different from survivors. A significantly higher frequency of dysstasia was also found in non-survivors, suggesting that the hemoconcentration seen in non-survivors is a result of dehydration as a complication of dysstasia, which causes difficulty in accessing drinking water. Dysstasia and hemoconcentration are typically observed in cows with fatal toxic mastitis [2]. These findings indicate that hemoconcentration can be considered a predictive characteristic for a poor prognosis and that positive intervention is needed to improve hemoconcentration and increased BUN concentrations in *E. coli* mastitis.

Our blood coagulation findings revealed decreased antithrombin activity and platelet counts, along with prolonged prothrombin time in non-survivors versus survivors. Decreased antithrombin activity and platelet counts [4] with prolonged prothrombin time [19] have been observed in patients with lethal DIC. In the initial stage of hemostasis, clotting inhibitors and factors, such as antithrombin and platelets, are utilized and eventually exhausted, if the trigger for coagulation persists, but can be fully compensated by the liver and bone marrow [14]. In human patients exhibiting decreased (>50%) antithrombin activity, a 96% mortality rate has been observed [5]. In the present study, prothrombin time was also negatively correlated with antithrombin activity and platelet count in survivors, but not in non-survivors. This finding suggests that in non-survivors, the compensability of the hemostasis system was lost. Therefore, the consumption coagulopathy in decompensated DIC can be considered as an important predictive finding associated with poor prognosis and mortality in cows with severe *E. coli* mastitis.

In summary, dysstasia associated with decreased antithrombin activity and platelet counts and with increased HCT and NEFA concentrations in dairy cows was considered to be the major diagnostic sign associated with a poor prognosis and high mortality after therapeutic treatment in acute *E. coli* mastitis.

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