Predictors of fatal outcomes resulting from acute *Escherichia coli* mastitis in dairy cows

Seiichi HAGIWARA¹, Kouichiru MORI² and Hajime NAGAHATA³*

¹Federation of Hokkaido Agricultural Mutual Aid Associations, North 4 West 1–1, Sapporo, Hokkaido 060–0004, Japan
²Okhotsk Agricultural Mutual Aid Association, Kitami, Hokkaido 090–0879, Japan
³Animal Health Unit, School of Veterinary Medicine, Rakuno Gakuen University, Bunkyo-dai, Ebetsu, Hokkaido 069–8501, Japan

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**ABSTRACT.** To evaluate the prognostic criteria for identifying cows at an increased risk of a fatal outcome from acute *Escherichia coli* mastitis, the potential cut-off values for five diagnostic parameters associated with a high mortality were determined by receiver operator characteristic curve analysis. These criteria were hematocrit value >32%, blood non-esterified fatty acid concentration >0.4 mEq/l, antithrombin activity <120%, platelet count <15 × 10⁵/ml and presence of dysstasia. Exceeding the cut-off values for at least three parameters on day 2 after onset predicted fatal outcome (predictive value 87.5%). When these prognostic criteria were applied to 34 clinical cases, cows that met three criteria were seven times more likely to die than cows that met fewer than three criteria.

**KEY WORDS:** bovine, *Escherichia coli* mastitis, fatal outcome, prognosis

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Acute coliform mastitis in lactating dairy cows is occasionally fatal. Endotoxemia [9], metabolic acidosis [2], uremia [1, 9] and increased aspartate aminotransferase (AST) activity [2, 13] are commonly observed in these cows. Bacteremia has been reported to occur in 32% [2, 14] to 75% [9] of cows with clinical coliform mastitis. Endotoxemia and disseminated intravascular coagulation in cows with acute *Escherichia coli* mastitis are the generally recognized causes of fatality [9].

To our knowledge, there are few reports on prediction of fatal acute *E. coli* mastitis [7, 15]. Significantly decreased milk production and high bacterial growth in the infected quarters are reported to predict fatal outcomes following experimental *E. coli* mammary infection [7]. Severe systemic signs include elevated rectal temperature, degree of enophthalmos, rumen contraction rate and signs of depression, which are reported to predict 48% of fatal outcomes resulting from clinical cases of coliform mastitis [15].

In our previous study, dysstasia associated with decreased antithrombin activity and platelet counts along with increased hematocrit (HCT) and blood non-esterified fatty acid (NEFA) concentrations were confirmed as prognostic parameters associated with a high mortality after therapeutic treatment of dairy cows with acute *E. coli* mastitis [6]. The ability of these five parameters to predict fatal outcomes following acute *E. coli* mastitis in dairy cows remains to be validated.

The aim of this study was to evaluate the predictors of fatal outcomes resulting from acute *E. coli* mastitis. In our present study, receiver operator characteristic (ROC) curve analysis was performed to identify potential cut-off values for the five parameters: dysstasia, antithrombin activity, platelet counts, HCT and NEFA.

Predictors of fatal outcomes resulting from acute *E. coli* mastitis were determined by ROC curve analysis of 24 Holstein dairy cows, which were fed in 17 dairy farms of Hokkaido, Japan. All 24 cows had an *E. coli* infection that was confirmed by the identification of the causative pathogen from infected quarter milk samples and one or more of the following findings on day 1: rectal temperature >40°C, heart rate >120 beats/min, respiratory frequency >30 breaths/min and blood total leukocyte counts <5,000/µl.

The cut-off values for differentiating between non-survivors and survivors amongst the 24 clinical cases were determined using data on dysstasia, HCT values, NEFA concentration, antithrombin activity and platelet counts. The sensitivity (proportion of non-survivors that were predicted to have a fatal outcome), specificity (proportion of survivors that were predicted to survive), predictive value (proportion of cows predicted to have a fatal outcome that were indeed non-survivors) and likelihood ratio (sensitivity/[1-specificity]) for non-survivors were determined for several ranges of the blood test parameters and dysstasia. ROC curves [sensitivity vs (1-specificity)] were constructed to identify the optimum threshold among the significant cut-off values. Odds ratios and 95% confidence intervals were determined to establish significant differences. A *P*-value of <0.05 was considered statistically significant.

The utility of the predictors was verified through their use in a trial on day 2 involving 34 clinical cases that were sampled similarly from 32 dairy farms in Hokkaido, Japan. The 34 cows had *E. coli* infection that had been confirmed
by isolation of bacteria from infected quarter milk samples and one or more of the following findings on day 1: rectal temperature >40°C, heart rate >120 beats/min and respiratory frequency >30 breaths/min. All of the tested bacterial isolates were sensitive in vitro to kanamycin as measured by antimicrobial susceptibility test using the disk diffusion method.

As the systemic antimicrobial therapy, kanamycin sulfate was administered to cows with acute E. coli mastitis on days 1, 2 and 3 after the onset. 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 intra-mammary infusion. Additionally, 1,000 U of heparin sodium (25–50 ml/cow/day), physiological saline solution (2,000–8,000 ml/cow/day) and 5% glucose (2,000–5,000 ml/cow/day) were intravenously administered when they were judged to be necessary. The frequency and dosages of medical treatments were identical for both survivors and non-survivors. On day 1, quarter milk [8] from affected cows was collected aseptically into sterilized culture tubes. E. coli isolates were identified using a specific biochemical system (Sysmex-bioMerieux Co., Ltd., Tokyo, Japan) and a laboratory-designed kit (VITEK 2; Sysmex-bioMerieux Co., Ltd., Tokyo, Japan) as an intra-mammary infusion. Additionally, 1,000 U of heparin sodium (25–50 ml/cow/day), physiological saline solution (2,000–8,000 ml/cow/day) and 5% glucose (2,000–5,000 ml/cow/day) were intravenously administered when they were judged to be necessary. The frequency and dosages of medical treatments were identical for both survivors and non-survivors. On day 1, quarter milk [8] from affected cows was collected aseptically into sterilized culture tubes. E. coli isolates were identified using a specific biochemical system (Sysmex-bioMerieux Co., Ltd., Tokyo, Japan) and a laboratory-designed kit (VITEK 2; Sysmex-bioMerieux Co., Ltd.) in a diagnostic laboratory (Kishimoto Medical Lab., Tomakomai, Japan). On day 2, 8–10 ml of 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). Analyses of the four serum parameters were conducted in a diagnostic laboratory (Kishimoto Medical Lab.) only on day 2 and not on day 1 or 3. 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) using plasma treated with sodium citrate. Serum NEFA concentration was measured using an automatic analyzer (JCA-BM2250; JEOL). HCT, platelet counts and white blood cell counts were measured with an automated instrument (Analyzer SE-9000; Sysmex) using plasma treated with EDTA.

There were 26 surviving cows (survivors) from among the 34 affected cows, with 8 cows euthanized (non-survivors) after unsuccessful therapeutic treatment. The cows were euthanized on days 4 (2 cows), 5 (3 cows), 6 (1 cow), 7 (1 cow) and 8 (1 cow). Among the 26 surviving cows, clinical symptoms resolved on days 3 (8 cows), 4 (7 cows), 5 (5 cows), 6 (2 cows), 7 (3 cows) and 9 (1 cow).

The sensitivity, specificity, predictive value and likelihood ratio for non-survivors were also determined for the 34 clinical cases. Odds ratio and 95% confidence interval around the odds ratio were determined to establish significant differences.

Cows with acute E. coli mastitis that met the prognostic criteria on day 2, comprising HCT >32%, NEFA >0.4 mEq/l, antithrombin activity <120%, platelet counts <15 × 10^4/ml and presence of dysstasia, were at higher risk of dying or being euthanized. If at least three of these parameters were met, a fatal outcome was predicted. From among the 24 clinical cases, cows with three or more parameters that exceeded the cut-off values were at a significantly (P<0.01) higher risk of death or euthanasia (Table 1). The fatal outcomes of the affected cows were predicted on day 2 (P<0.01) and day 3 (P<0.01), but not on day 1 (P=0.06). The sensitivity, specificity, predictive value and likelihood ratio of the prognostic criteria on days 2 and 3 were 100%, 94.1%, 87.5% and 17, and 100%, 83.3%, 75.0% and 6, respectively (Table 1).

When these criteria and cut-off values were applied to 34 other clinical cases, cows with three or more of the parameters that met the criteria on day 2 were seven times more likely to die compared with cows with fewer than three of the parameters that met the criteria on day 2 (P<0.05, Table 2). The sensitivity, specificity, predictive value and likelihood ratio of the prognostic criteria for the cows on day 2 were 62.5%, 80.8%, 50.5% and 3.3, respectively (Table 2).

When the predictive value of each of these five parameters was considered as an individual prognostic criterion, dysstasia was the most reliable parameter for diagnosing fatal outcomes resulting from acute E. coli mastitis. Except for day 1, among these 34 cows, those with dysstasia were at a significantly higher risk of dying or being euthanized (P<0.01) (Tables 1 and 2). The fatal outcomes resulting from acute E. coli mastitis could be predicted on day 2 after onset. Cows showing severe clinical signs for three consecutive days after onset of naturally occurring acute E. coli mastitis were found to be at a significantly higher risk of dying or being culled [11]. Cows showing severe systemic signs for four consecutive days after experimentally induced E. coli mammary infections were euthanized [7], whereas cows with no signs on day 2 after infection recovered [7, 10]. Significantly increased HCT and NEFA concentrations, and significantly decreased antithrombin activity and platelet counts were observed in non-survivors, compared with survivors on day 2 and day 3, but not on day 1 [6]. The differences in clinical symptoms between non-survivors and survivors became apparent starting from day 2 after the onset of acute E. coli mastitis.

Increased HCT and NEFA concentrations were associated with a higher risk of a fatal outcome from acute E. coli mastitis. Non-surviving cows showed higher HCT (39%) than that of surviving cows (35%) in clinical acute coliform mastitis [2]. Severe symptoms were observed in cows with higher NEFA (0.49 mEq/l) prior to experimental intramammary E. coli infection, compared with cows with lower NEFA (0.31 mEq/l) [12].

Decreased antithrombin activity and platelet counts [4] have been observed in patients with lethal disseminated intravascular coagulation. In human patients exhibiting decreased (<50%) antithrombin activity, a 96% mortality rate has been observed [5]. Platelet count was also an accurate indicator of mortality in human sepsis; significantly decreased platelet count (17.4 × 10^4/ml) has been observed in non-survivors compared with survivors (26.8 × 10^4/ml). [3]

Dysstasia may be a major parameter for predicting fatal outcomes resulting from acute E. coli mastitis. Recumbency
at 2 days after experimental *E. coli* mammary infection was reported as a common clinical finding in two cows that were euthanized because of severe clinical mastitis [7]. Of the 56 cows, a diagnosis of dysstasia at the first clinical examination was made in 12 with toxic clinical mastitis, with 31 eventually slaughtered or euthanized [1]. Dysstasia was the most reliable predictor of a fatal outcome in this study (Tables 1 and 2). Examinations focused on dysstasia may help to predict fatal outcomes resulting from acute *E. coli* mastitis. This study verified that fatal outcomes for dairy cows with acute *E. coli* mastitis can be accurately predicted using specific prognostic criteria and cut-off values of HCT >32%, NEFA >0.4 mEq/l, antithrombin activity <120% and platelet counts <15 × 10⁴/ml, along with the presence of dysstasia. The usefulness of these prognostic criteria and cut-off values was verified in cows with fatal *E. coli* mastitis on dairy farms.

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### Table 1. Associations of hematocrit, non-esterified fatty acid, antithrombin activity, platelet count and dysstasia with the fatal outcome of naturally occurring acute *Escherichia coli* mastitis in 24 clinical cases

| Parameters                      | Cutpoint | Odds ratio a)   | 95% CI a) | P a) | Sensitivity (%) | Specificity (%) | Predictive value (%) | Likelihood ratio |
|---------------------------------|----------|-----------------|-----------|------|----------------|------------------|---------------------|-----------------|
| Measured at onset (Day 1, n=24) | Hematocrit (%) >32 | 3.3  | 0.5 to 22.9  | 0.24 | 50.0  | 76.5  | 42.9  | 2.1  |
|                                 | Non-esterified fatty acid (mEq/l) >0.4 | 4.8  | 0.7 to 35.2  | 0.13 | 66.7  | 70.6  | 44.4  | 2.3  |
|                                 | Antithrombin activity (%) <120 | ...  | ...         | ...  | 14.3  | 100.0 | 100.0 | ...  |
|                                 | Platelet count (×10⁴/ml) <15 | 0.8  | 0.1 to 5.0  | 0.60 | 42.9  | 52.9  | 27.3  | 0.9  |
|                                 | Dysstasia positive | ...  | ...         | ...  | 71.4  | 100.0 | 100.0 | ...  |
| At least 3 parameters fell within the ranges | 12.0 | 1.0 to 148.3  | 0.06 | 42.9  | 94.1  | 75.0  | 7.29 |

| Parameters                      | Cutpoint | Odds ratio a)   | 95% CI a) | P a) | Sensitivity (%) | Specificity (%) | Predictive value (%) | Likelihood ratio |
|---------------------------------|----------|-----------------|-----------|------|----------------|------------------|---------------------|-----------------|
| Measured in one day after onset (Day 2, n=24) | Hematocrit (%) >32 | ...  | ...         | ...  | 71.4  | 100.0 | 100.0 | ...  |
|                                 | Non-esterified fatty acid (mEq/l) >0.4 | ...  | ...         | ...  | 100.0 | 62.5  | 53.8  | 2.7  |
|                                 | Antithrombin activity (%) <120 | 10.0  | 1.2 to 81.8  | 0.04 | 57.1  | 88.2  | 66.7  | 4.9  |
|                                 | Platelet count (×10⁴/ml) <15 | 3.6  | 0.5 to 24.0  | 0.19 | 71.4  | 58.8  | 41.7  | 1.7  |
|                                 | Dysstasia positive | ...  | ...         | ...  | 100.0 | 100.0 | 100.0 | ...  |
| At least 3 parameters fell within the ranges | ...  | ...         | ...  | 100.0 | 94.1  | 87.5  | 17   |

| Parameters                      | Cutpoint | Odds ratio a)   | 95% CI a) | P a) | Sensitivity (%) | Specificity (%) | Predictive value (%) | Likelihood ratio |
|---------------------------------|----------|-----------------|-----------|------|----------------|------------------|---------------------|-----------------|
| Measured in two day after onset (Day 3, n=18) | Hematocrit (%) >32 | ...  | ...         | ...  | 100.0 | 91.7  | 85.7  | 12.0 |
|                                 | Non-esterified fatty acid (mEq/l) >0.4 | ...  | ...         | ...  | 100.0 | 58.3  | 54.5  | 2.4  |
|                                 | Antithrombin activity (%) <120 | ...  | ...         | ...  | 100.0 | 75.0  | 66.7  | 4.0  |
|                                 | Platelet count (×10⁴/ml) <15 | ...  | ...         | ...  | 83.3  | 58.3  | 50.0  | 2.0  |
|                                 | Dysstasia positive | ...  | ...         | ...  | 100.0 | 100.0 | 100.0 | ...  |
| At least 3 parameters fell within the ranges | ...  | ...         | ...  | 100.0 | 83.3  | 75.0  | 6    |

### Table 2. Associations of hematocrit, non-esterified fatty acid, antithrombin activity, platelet count and dysstasia with the fatal outcome of naturally occurring acute *Escherichia coli* mastitis in 34 clinical cases

| Parameters                      | Cutpoint | Odds ratio a)   | 95% CI a) | P a) | Sensitivity (%) | Specificity (%) | Predictive value (%) | Likelihood ratio |
|---------------------------------|----------|-----------------|-----------|------|----------------|------------------|---------------------|-----------------|
| Observed at onset (Day 1, n=34) | Dysstasia positive | 1.8  | 0.3 to 12.5  | 0.44 | 25.0  | 84.6  | 33.3  | 1.6  |
| Observed in one day after onset (Day 2, n=34) | Hematocrit (%) >32 | 4.5  | 0.8 to 24.1  | 0.08 | 62.5  | 73.1  | 41.7  | 2.3  |
|                                 | Non-esterified fatty acid (mEq/l) >0.4 | 3.2  | 0.6 to 16.3  | 0.16 | 62.5  | 65.4  | 35.7  | 1.8  |
|                                 | Antithrombin activity (%) <120 | 2.3  | 0.4 to 11.6  | 0.28 | 62.5  | 57.7  | 31.3  | 1.5  |
|                                 | Platelet count (×10⁴/ml) <15 | 12.6  | 1.9 to 82.1  | <0.01 | 75.0  | 80.8  | 54.5  | 3.9  |
|                                 | Dysstasia positive | 12.8  | 2.0 to 82.9  | <0.01 | 62.5  | 88.5  | 62.5  | 5.4  |
| At least 3 parameters fell within the ranges | 7.0  | 1.2 to 39.6  | <0.05 | 62.5  | 80.8  | 50.5  | 3.3  |

| Parameters                      | Cutpoint | Odds ratio a)   | 95% CI a) | P a) | Sensitivity (%) | Specificity (%) | Predictive value (%) | Likelihood ratio |
|---------------------------------|----------|-----------------|-----------|------|----------------|------------------|---------------------|-----------------|
| Observed in two day after onset (Day 3, n=24) | Dysstasia positive | 21.0 | 2.4 to 185.9 | <0.01 | 75.0 | 87.5 | 33.3 | 6.0 |

a) From Fisher’s exact probability test. 95% CI=95% confidence interval around the odds ratio. b) Odds ratio, 95% CI and likelihood were not calculated, because none of surviving cows was less than the cutpoint. c) Odds ratio and 95% CI were not calculated, because all of these values of non-surviving cows were included a range designated by each cutpoint.
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