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Classification of acute clinical mastitis on the base of vital signs and complete blood count test in dairy cows

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Abstract : This study was carried out to evaluate the diagnosis of acute clinical mastitis (ACM) which was based on the vital signs and complete blood count (CBC) tests in dairy cows. Twenty eight dairy cows diagnosed with ACM, were selected for the study between Jan 2003 and July 2006 in the National Institute of Animal Science. Based on their vital signs (rectal temperature, depression, rumen contraction and, dehydration status), ACM was divided into three different classes; mild, moderate and severe forms. In addition, ACM cows were subjected to CBC tests for further diagnosis of ACM. Of the 27 dairy cows diagnosed with ACM, 3 cows were determined to have a mild form, while moderate and sever forms were each observed in twelve cows. Among of them, 4 cows died, 5 cows were culled and 18 cows were recovered. In the mild form, all haematological parameters were comparable with normal values. However, leukopenia, due to neutropenia and lymphocytopenia, appeared characteristically in the moderate and severe forms. Using the observation of vital signs in conjunction with CBC tests, the diagnosis of ACM is more accurate, and is helpful in making decisions of whether treatment or culling of dairy cows infected with ACM is most appropriate.

Keywords : acute clinical mastitis, complete blood count test, vital signs, dairy cow

Introduction

Clinical mastitis results when the cow's immune system responds with enough intensity to cause an intramammary infection (IMI), and to elicit signs of inflammation that are physically observable. The nearly universal observation of clinical mastitis is an abnormal color or texture of the milk. As the degree of local inflammation increases, swelling, discoloration, and pain in the udder may become evident. In mild cases of clinical mastitis, clinical signs are generally restricted to local involvement of the udder, but in severe cases of clinical mastitis there is systemic involvement, including depression, fever, shock, and recumbency. Especially in severe cases, milk production can be decreased profoundly, and the affected quarter may become agalactic, in which case culling may be eventually needed, or in a small proportion of cases, death may result [6, 12].

Acute clinical mastitis (ACM) is often characterized by swollen, painful glands that may be edematous or very hard, making it difficult for the patient to walk normally [5, 11, 13]. Systemic signs are presented, depending on the type of clinical mastitis, and its intensity may vary from mild to severe forms suddenly. Systemic signs of mild/moderate/

severe ACM include anorexia, depression, decreased rumen motility and elevated rectal temperature [1]. The severity of infection determines the degree of production loss and the final outcome. If they remain untreated could be lead to death and culling, and therefore, a rapid cow-side method for evaluating the severity of ACM may prove useful in determining early appropriate treatment and management strategies to reduce production and animal losses.

The purpose of this study is to report the incidence of clinical mastitis in dairy cows and to establish a new classification for ACM by using the most appropriate diagnostic tool. The present study may assist with the development of a strategy for the early treatment of clinical mastitis, restoring heavy production losses.

Materials and Methods

Animals

Lactating cows at the National Institute of Animal Science dairy research farm that were diagnosed with acute clinical mastitis between Jan 2003 and July 2006 were eligible for inclusion in the study. At the time of the investigation period, the herd contained approximately 200 lactating Holstein

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cows. The mean somatic cell count for the herd's milk ranged from 150,000 to 300,000 cell/mL. Cows were fed a total mixed ration based on production levels and were brought to parlor 2 times a day. All cows were housed in free stalls and were not vaccinated against bovine mastitis.

Farm personnel identified cows as possibly having ACM if one or more of following signs were present: abnormal milk production, sudden drop in milk production, udder redness and hardness, pain during milking, and swelling of quarter. Cows of the study were then diagnosed by a veterinarian.

The cows had been examined at the time that ACM had initially been identified (time 0), and rectal temperature, heart rate, rumen contraction rate, dehydration, and attitude had been recorded, along with physical characteristic of the affected mammary glands (firmness and swelling) and viscosity of the secretion.

Treatment of ACM cows was done according to medical protocol, and medication after physical and clinical examination included antibiotics, anti-inflammatory drugs and supportive measurements until the cows recovered. If systemic and local clinical signs recovered, treatment was stopped. Age, lactation number, days in milk (DIM), rumen contraction rate, rectal temperature, and dehydration status were recorded.

Classification of acute clinical mastitis

According to Wenz *et al.* [14], cows with ACM were classified as having mild, moderate or severe forms on the basis of clinical parameters, including rectal temperature, degree of dehydration, degree of rumen contraction and depression at the initial observation of cows with clinical mastitis.

Temperature was classified on the basis of severity of rectal temperature as 0 (37.8~39.3°C), 1 (39.3~39.8°C), and 2 (>39.8 or <37.8°C). Rumen contractions were classified with rumen motility as 0 (≤ 2 /min), 1 (1/min), and 2 (0/min). Dehydration status was estimated on the basis of the degree of enophthalmos and was scored as 0 (none), 1 (mild enophthalmos), and 2 (marked enophthalmos). Depression was classified on the basis of appetite as 0 (none), 1 (mild), and 2 (marked).

Scores assigned for each parameter were arbitrary, but reflected the increasing severity of the specific disease sign based on clinical impressions. Cows with total score of 0 to 2 were classified as having mild forms of ACM, cows with total score of 3 to 5 were classified as having moderate forms of ACM, and cows with total score of 6 to 8 were classified as having severe forms of ACM.

CBC test

Blood samples were collected from the jugular vein at the first observation of cows with ACM and the following treatment. The physical status of cows with ACM was examined during treatment. Hematologic values were measured using a multi-species blood counter (Hema-Vet 850; CDC tech, USA).

Outcome

The survival and retention of the animals in the study group was used to determine the outcome. Cows that died during the episode of mastitis were classified dead. Cows leaving the herd as a direct result of mastitis for 30 days after the episode of mastitis were classified as culled. Cows were present even after episode of mastitis were classified as retained.

Data analysis

Normally distributed continuous variables were compared among groups (mild, moderate, or severe form) by means of ANOVA. Categorical variables (dehydration, attitude, proportion of cows with CBC test) were compared among groups by means of χ^2 test. For all statistical tests, a value of $p < 0.05$ was considered to be significant.

Results

Severity classifications and outcome

For cows identified as having acute clinical mastitis, all of the initial vital signs, including body temperature, depression, rumen motility and dehydration were significantly higher than those of animals with mild and moderate forms of ACM (Table 1). However, clinical vital signs were not found to be different between mild and moderate forms of ACM.

Of the 27 cows in the study, 4 cows died and 5 cows were culled. Of them, 44.4% of cows had each of moderate and severe forms of ACM, and the remaining (11.1%) cows had mild ACM (Table 2) as determined by the initial inspection of vital signs. The percentage of cows classified as having ACM was significantly higher for the animals suspected as having moderate and severe forms, than for those suspected as having mild forms ($p < 0.05$).

The mean parity of cows with mild, moderate, and severe ACM were 2.5, 2.3, and 2.2 respectively. The maximum number (13) of test cows were in second parity which was

Table 1. Vital sign score at first event day classified by the severity of acute clinical mastitis (ACM)

Vital sign	Classification of ACM		
	Mild	Moderate	Severe
Temperature	0.4 ^a	0.7 ^a	1.8 ^b
Depression	0.8 ^a	0.8 ^a	1.8 ^b
Rumen motility	0.4 ^a	0.8 ^a	1.6 ^b
Dehydration	0 ^a	0.4 ^a	1.1 ^b

Values within a row with different superscripts are significantly ($p < 0.05$) different. Temperature: 0 (37.8~39.3°C), 1 (39.3~39.8°C), and 2 (>39.8 or <37.8°C). Depression: 0 (none), 1 (mild), and 2 (marked). Rumen motility: 0 (≤ 2 /min), 1 (1/min), and 2 (0/min). Dehydration status: 0 (none), 1 (mild enophthalmos), and 2 (marked enophthalmos). Mild forms (mild ACM): cows with total score of 0 to 2. Moderate forms (moderate ACM): cows with total score of 3 to 5. Severe forms (severe ACM): cows with total score of 6 to 8.

Table 2. The outcome of cows with ACM classified on the basis of disease severity

Class	No. of cows (%)			
	Total (%)	Recover	Culling	Death
Mild	3 (11.1)	3		
Moderate	12 (44.4)	8	1	2
Severe	12 (44.4)	7	4	2
Total	27 (100.0)	18 (66.6)	5 (18.5)	4 (14.8)

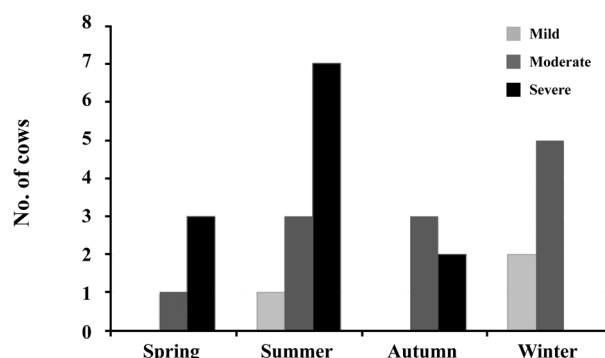
Table 3. Distribution of dairy cows with ACM by days in milk

Class	No. of cows	Days in milk				
		0~30	31~60	61~90	91~150	151~300
Mild	3				1	2
Moderate	12	10			1	1
Severe	12	6	1		1	4
Total	27	16	1	0	3	7

followed by the third (7) and first (5), with only two cows in the 4th, or higher, parity.

The maximum number of cases (16 cows) occurred during the first 30 DIM and 30% cases occurred over mid lactation period (Table 3).

Moderate cases tended to occur in the summer and winter seasons, but severe ACM mainly occurred during the summer season from May to October (Fig. 1). The duration of

**Fig. 1.** Distribution of the 27 acute clinical mastitis cases of dairy cows according to season.

treatment in cases of cows with ACM varied from 1 day to 18 days.

CBC test

Samples were collected from twenty seven cows diagnosed with ACM. Seven cows were excluded from the study because necessary data necessary for the study was missing. Differences among groups that were deemed to clinical important were found for total WBC, neutrophil, lymphocyte, eosinophil, basophil, RBC and platelet count (Table 4).

Median values of total WBC, neutrophil, eosinophil, basophil and platelet count at day 0 were significantly lower for moderate and severe cases than for cases of mild ACM. The median value of lymphocytes at day 0 was significantly ($p <$

Table 4. Results of complete blood count (CBC) test at the time of initial identification of cow with ACM (day 0) classified on the basis of disease severity

Variable	Day	Class of ACM		
		Mild (n = 3)	Moderate (n = 9)	Severe (n = 8)
WBC ($\times 10^3/\mu\text{L}$)	0	10.76 ^a (9.38~14.14)	4.87 ^b (1.5~10.26)	3.06 ^b (1.32~5.6)
	1	12.78 (7.12~18.84)	11.71 (3.20~22.66)	6.77 (1.94~13.6)
Neutrophils ($\times 10^3/\mu\text{L}$)	0	6.39 ^a (0.34~10.9)	2.35 ^b (0.51~6.88)	1.28 ^b (0.23~2.82)
	1	9.65 (4.04~15.06)	8.45 (2.59~20.31)	3.73 (0.51~7.72)
Lymphocytes ($\times 10^3/\mu\text{L}$)	0	3.59 ^a (1.86~8.80)	1.84 ^{ab} (0.8~3.08)	1.43 ^b (0.78~3.47)
	1	1.99 ^a (1.87~2.19)	3.46 ^b (2.46~4.26)	2.11 ^a (1.16~4.17)
Eosinophils ($\times 10^3/\mu\text{L}$)	0	0.40 ^a (0.03~0.67)	0.15 ^b (0.08~0.33)	0.10 ^b (0~0.33)
	1	0.71 (0.12~1.19)	0.34 (0.07~0.95)	0.66 (0.02~1.98)
Basophil ($\times 10^3/\mu\text{L}$)	0	0.07 ^a (0.02~0.18)	0.02 ^b (0~0.06)	0.01 ^b (0~0.04)
	1	0.07 (0.03~0.12)	0.05 (0~0.12)	0.04 (0~0.10)
RBC ($\times 10^6/\mu\text{L}$)	0	6.92 ^a (5.47~8.08)	7.64 ^{ab} (5.35~9.62)	8.78 ^b (6.58~11.3)
	1	6.73 (6.06~7.13)	7.89 (5.94~11.25)	8.97 (6.56~13.12)
Platelet ($\times 10^3/\mu\text{L}$)	0	422 ^a (190~666)	211 ^b (61~291)	211 ^b (107~445)
	1	488 ^a (283~766)	274 ^b (224~311)	167 ^b (68~445)

Values are expressed as median (ranges). Values within a row with different superscripts are significantly ($p < 0.05$) different.

Table 5. Change of mean value of blood parameters at the initial day (0) and day 1 according to the outcome

Parameters		Outcome of cows with ACM		
		Recover (n = 18)	Cull (n=5)	Death (n=4)
RBC ($\times 10^6/\mu\text{L}$)	0	7.90 \pm 1.21	7.16 \pm 1.57	9.31 \pm 2.49
	1	7.72 ^a \pm 1.75	6.99 ^a \pm 1.93	11.07 ^b \pm 2.38
Hemoglobin (g/dL)	0	7.53 ^a \pm 1.31	10.46 ^b \pm 1.58	11.9 ^b \pm 1.47
	1	10.13 ^a \pm 1.59	9.25 ^a \pm 1.52	13.5 ^b \pm 1.63
Hematocrit (%)	0	35.9 \pm 4.25	33.4 \pm 5.79	41.5 \pm 4.94
	1	34.4 ^a \pm 3.85	33.1 ^a \pm 4.63	49.37 ^b \pm 3.65

Values are expressed as Mean \pm SD. Values within a row with different superscripts are significantly ($p < 0.05$) different.

0.05) lower in severe cases than in moderate and mild cases of ACM. The median values of RBC count at day 0 were significantly ($p < 0.05$) higher in severe cases than in moderate and mild cases of ACM. The median value of lymphocyte counts at day 1 was significantly ($p < 0.05$) higher in moderate cases than in mild and severe cases of ACM. The median value of RBC at day 1 was higher in severe cases of ACM but this difference was not found to be significant. Platelets were significantly higher ($p < 0.05$) at day 0 and 1 in mild cases than in moderate and severe cases of ACM.

The changes of blood parameters were analyzed according to the results of the ACM outcome (Table 5). Hemoglobin (HB) at the first day was significantly ($p < 0.05$) higher in the cull and death groups. However, RBC, Hematocrit (HCT) and HB at day 1 was significantly ($p < 0.05$) higher in the death group, when compared to all other groups.

Discussion

Results of the present study suggest that a classification scheme based on readily observable systemic disease signs can be used to classify disease severity in cow with ACM. In the present study of naturally occurring ACM, severity of systemic disease signs was greater in cows with severe ACM than those with mild and moderate ACM. For cows grouped according to this classification scheme, significant differences in clinicopathologic findings and outcomes were identified according to the outcome of the disease. A few cows diagnosed ACM were recumbent at the initiation of antimicrobial therapy and a few cows were not eating 24 h later; however, 62.9% of these cows recovered.

In the present study, the degree of derangement of these parameters was related to the severity of systemic disease signs. Wenz *et al.* [12] suggest that bacteremia develops in a substantial proportion of cows with ACM, and that the classification of the severity of disease is important for the establishment of effective treatment protocols. This suggests that certain physiologic derangements may be associated with the outcome of cows with ACM.

Aggressive antibiotic treatment has been shown to improve clinical cure rates and to reduce the severity of clinical dis-

eases in cows with ACM [3, 8]. However, treatment of cows with ACM remains controversial, and studies of antibiotic efficacy have yielded conflicting results [9]. Factors that may influence efficacy include whether the selected antibiotic has an appropriate duration and route of antibiotic administration, as well as the disease severity [6]. The mean lactation period of mild, moderate and severe cases were 92, 25, and 75 days, respectively. The mean lactation number and lactation period were not significantly different among the three groups.

The overall percentage of cows that died during, or were culled within 30 days after the episode of mastitis increased significantly as disease severity increased. Bradley and Green [2] reported that mastitis due to *Escherichia coli* was more severe than mastitis due to other causes, and that it tended to be more severe in early lactation and during the housing period. Mastitis was significantly more severe (grade 2 and 3) in the herd with the lowest bulk milk somatic cell count.

Morin *et al.* [8] confirmed that clinical observations were not sufficiently accurate to be useful for predicting gram-negative mastitis, but other researchers [5, 12] suggested that the season of the year, stage of lactation, and a classification scheme based on readily observable systemic disease signs can be used to classify severity in cows with acute coliform mastitis.

Analysis of results of clinicopathologic testing in these cows demonstrated that severity classifications obtained on the basis of systemic disease related well to less readily measurable but physiologically important changes in cows with ACM.

Our study is in agreement with previous studies of cows with experimentally induced and naturally occurring ACM, for which leukopenia, neutropenia, lymphopenia have been reported [3, 7]. Smith *et al.* [10] recommended that routine hematological analysis is useful in guiding initial treatment decisions, particularly in valuable cows or when cows are hospitalized. It is possible that the proposed severity classification system could have been refined by including other parameters, such as neutrophil count and RBC count at the initial assessment.

In this study, RBC, HCT and HB at day 1 was signifi-

cantly ($p < 0.05$) higher in the death group than in the others. This was consistent with the result of Green *et al.* [4] who found that there was a significant difference between the mean PCV of the cows which survived and those which died. Also, there were significant differences in rectal temperatures, eyelid skin tent time and packed cell volume between the cows which survived and those which died [4].

We found a positive relationship between the severity of systemic disease signs and the percentage of cows with an undesirable outcome (death or culling). The classification scheme on the basis of vital signs is simple, rapid, and can be used on the farm to provide immediate information that should prove valuable in determining appropriate treatment and risk of an undesirable outcome for cows with ACM.

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