PEER REVIEWED

Hematologic Abnormalities over 5 Days in Cows with Acute *Escherichia coli* Mastitis

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Abstract

Hematologic data from 45 adult Holstein cows with coliform mastitis and systemic clinical signs were studied retrospectively. They developed panleukopenia that resolved over several days, first through an increase in immature forms and later by an increase in mature neutrophils. Neutropenia lasted three days in most cows. The majority of cows in early lactation did not develop neutropenia for an unknown reason. Cows with fatal disease could not be distinguished based on hematologic analysis, because most died early in the disease, while both surviving and non-surviving cows still had panleukopenia. Cows with coliform bacteremia had prolonged neutropenia and higher metamyelocyte and myelocyte counts during the regenerative phase. These findings suggest that hematologic analysis during the regenerative phase may be useful to identify cows with bacteremia for more aggressive treatment.

Introduction

Acute coliform mastitis is an important disease for the individual cow, and can cause partial or complete agalactia, abortion, or death.[7] It is also an important disease for the dairy industry as a whole, with an annual incidence of 20 cases per 100 cows or higher.[5] Although vaccination of cattle with a mutant *Escherichia coli* bacterin reduces the incidence of clinical coliform intramammary infections[8] and reduces the severity of clinical signs seen after experimental intramammary inoculation,[13] severe coliform mastitis can occur in both vaccinated and unvaccinated cows. Therefore, information on the assessment and treatment of individual cows with acute coliform mastitis remains important.

Most information on the pathogenesis of coliform mastitis is from cattle experimentally inoculated with *E. coli* or endotoxin. We recently reported that cows with naturally occurring acute coliform mastitis with systemic signs appear to differ in several important ways from cattle with experimentally induced disease.[2] These differences include neutropenia and the presence of bacteria in the mammary gland several days after infection becomes apparent, the presence of metabolic abnormalities including acidosis and azotemia in non-surviving cows, and the previously unreported high incidence of bacteremia in cows with the severe form of this disease. These differences support the importance of studying cattle with naturally occurring disease when investigating pathogenesis.

It has been suggested that characteristic hematologic abnormalities can be useful in the diagnosis of acute coliform mastitis and that worsening of these abnormalities with time indicates a poor prognosis.[22] Leukopenia characterized by both neutropenia and lymphopenia is commonly seen in cows with naturally occurring acute coliform mastitis[2,21] and also occurs after experimental inoculation of the mammary gland with either *E. coli*[9] or endotoxin.[1] These abnormalities are very transient in cows with experimental disease,[1,9] and their persistence with natural disease is likely an important, but previously overlooked finding. Previous reports suggest that hematologic data acquired at the time of hospitalization do not differ between cattle that survive or do not survive infection,[2,4] but appear to provide information concerning bacteremia.[2] However, these reports group cattle that had shown disease signs for varying lengths of time. In this report, we investigated the diagnostic value of hematologic abnormalities from cattle with naturally occurring acute coliform mastitis, with particular emphasis on the changes in the leukogram at different stages of the disease.

Materials and Methods

Study Population—Medical records were reviewed from 45 Holstein cows with coliform mastitis and clinical signs of systemic illness admitted to the Veterinary Teaching Hospital at Colorado State University between January 1, 1990 and June 30, 1996. This included all Holstein cows admitted over the period of the study with clinical signs of acute coliform mastitis, hematologic data, and a positive milk culture for *E. coli*. Hematology data (CBC), date of blood collection, and date of onset of clinical signs of coliform mastitis were retrieved from the medical records. Hematology data were grouped by time elapsed since the onset of clinical signs, regardless of day of admission to the hospital. By this method, day 1 refers to blood drawn within 24 hours of onset of signs, day 2 within 48 hours, and so forth. Data from the first 5 days of illness were used in this report. Initial CBC samples were taken from 45 cows on admission, and 42 repeat samples were taken, with up to 4 samples from an individual cow. Differential counts of mature neutrophils, band cells, metamyelocytes, myelocytes, and lymphocytes were determined manually as a percentage of the total white blood cell count, which was determined by an automated cell counter.[a] Mature neutrophils were defined as cells with a thin, irregularly pinched nucleus and without strongly staining cytoplasmic granules. Band cells were defined as similar cells with a thicker, smooth-walled nucleus that did not appear to be indented at any point. Metamyelocytes were defined as similar cells with an ovoid nucleus with a single indentation and a light, basophilic cytoplasm. Myelocytes were defined as a slightly larger, similar staining cell with a round to slightly indented nucleus and a granular cytoplasm.

Cattle were grouped as non-survivors if they died in spite of treatment or were euthanized for humane reasons during unsuccessful treatment. No cattle were euthanized based on unwillingness of the owner to continue the recommended treatment, which consisted of oral and intravenous fluids, flunixin meglumine injections, frequent stripping of affected quarters, and parenteral antibiotic administration. Cattle were grouped as bacteremic if *E. coli* was isolated from a single blood culture sample taken on admission to the clinic. They were grouped as non-bacteremic if this culture sample was negative and excluded from that part of the study if no culture sample was obtained.

Statistical Analysis—As a preliminary test, Day 2 CBC results from cattle newly admitted to the clinic (initial CBC) were compared by the Mann-Whitney U test to results from cattle already under clinic care (repeat CBC). This procedure was repeated for days 3 through 5 and after these tests revealed no significant differences, repeat and initial data were pooled for each day in all subsequent analyses. For comparison, the cows were stratified in 3 separate groups: 1) surviving cows were compared to non-surviving cows, 2) bacteremic cows were compared to non-bacteremic cows, and 3) cows in the first 28 days of lactation were compared to cattle in later lactation. Time points containing less than 3 cows for a group were not reported or used in statistical analyses.

Based on preliminary evaluation of the data by determination of Pearson's coefficient of skew for each sample group, non-parametric methods were used for statistical analysis. Changes over time in each group for each cell type were assessed with the Kruskal-Wallis one-way ANOVA. Groups for which a significant difference was found were compared on a day-to-day basis with the Mann-Whitney U test. The Mann-Whitney U test also was used to compare cell counts on each day between the groups outlined above. For all tests, statistical significance was determined when $P < .05$.

Results

Sample medians are compiled in Tables 1a-e. Mean values have been included and demonstrate that mean values were higher than median values in 145 of 160 determinations. There were between 3 and 19 samples for each determination.

The typical hematologic abnormalities seen in these cows with acute coliform mastitis with systemic signs can be summarized as follows: most cows had neutropenia for 72 hours and lymphopenia for at least 5 days. Neutrophil counts were lower on the second day than the first, although this difference was not significant. Immature neutrophil counts, made up mainly of band cells and metamyelocytes, were initially low, then increased on the third and fourth days of illness, and finally decreased after mature neutrophil counts had become normal. Myelocytes were seen rarely. This same general pattern could be applied to the subgroups of non-bacteremic cows and surviving cows.

Bacteremic cows had significantly lower segmented and band neutrophil counts on day 4 than non-bacteremic cows, and did not regain normal neutrophil counts until day 5. Additionally, bacteremic cows had higher metamyelocyte counts on day 2 and myelocyte counts on day 4 ($P < .05$). There were not enough bacteremic cows to report data on day 1. There were no significant differences between surviving and non-surviving cows for days 1 to 3, and not enough non-surviving cattle to report data after the third day of illness.

Cattle in the first 4 weeks of lactation typically did not develop neutropenia, and had high counts of band neutrophils sooner than cattle further into lactation. The difference in segmented neutrophil counts for the first 3 days approached significance ($P = .053$

[a]Coulter S+ IV; Coulter Corp, Hialeah, FL

Table 1. Median (and mean) peripheral blood nucleated cell counts from cows with acute coliform mastitis. All counts are expressed in cells per μL.

a. Neutrophils (reference range: 600 to 4000 cells/μL)

Day	All Cows	Cows with Bacteremia	Cows without Bacteremia	Non-Survivors	Survivors	Early Lactation	Mid/Late Lactation
1	400[a]	id	300[a,b]	400	350[a]	800	250[a]
	(601)		(579)	(927)	(485)	(1137)	(354)
	n = 19		n = 12	n = 5	n = 14	n = 7	n = 12
2	270[a]	300	270[a]	185	300[a]	950	205[a]
	(890)	(1629)	(625)	(822)	(910)	(1695)	(552)
	n = 27	n = 8	n = 15	n = 6	n = 21	n = 8	n = 19
3	500[a]	350	400	800	400[a]	800	400[a,b]
	(1451)	(2046)	(950)	(1702)	(1350)	(3079)	(636)
	n = 21	n = 11	n = 8	n = 6	n = 15	n = 7	n = 14
4	850	250†	2050[b,c]†	id	900	2600	900[b,c]
	(1608)	(275)	(2450)		(1709)	(1933)	(1500)
	n = 12	n = 4	n = 4		n = 11	n = 3	n = 9
5	1850[b]	700	2100[c]	id	2100[b]	3100	1000[c]
	(2150)	(1467)	(2200)		(2229)	(2500)	(1800)
	n = 8	n = 3	n = 3		n = 7	n = 4	n = 4

b. Band Cells (reference range: 0 to 100 cells/μL)

Day	All Cows	Cows with Bacteremia	Cows without Bacteremia	Non-Survivors	Survivors	Early Lactation	Mid/Late Lactation
1	200[a]	id	100[a]	400	165[a]	400†	100[a]†
	(366)		(189)	(305)	(388)	(802)	(165)
2	200[a]	700	200[a]	190	200[a]	1150†	150[a]†
	(1130)	(3257)	(355)	(1363)	(1064)	(3063)	(317)
3	600[b]	650	750[b]	400	700[b]	1100	500[b]
	(1274)	(1605)	(1038)	(1035)	(1369)	(2234)	(794)
4	2150[c]	850	3300[c]	id	2400[b]	1900	1600[c]
	(2385)	(1725)	(3429)		(2520)	(1733)	(2602)
5	1250[b,c]	1200	1300[b,c]	id	1300[b]	1600	1300[c]
	(1538)	(2133)	(1333)		(1714)	(1825)	(1250)

c. Metamyelocytes (reference range: 0 cells/μL)

Day	All Cows	Cows with Bacteremia	Cows without Bacteremia	Non-Survivors	Survivors	Early Lactation	Mid/Late Lactation
1	60[a,b]	id	14[a,b]	0	100[a]	120	0[a]
	(101)		(67)	(23)	(129)	(140)	(83)
2	100[a,c]	500†	100[a,c]†	50	100[a,b]	150	50[a,b]
	(581)	(1686)	(239)	(1483)	(323)	(1225)	(309)
3	400[d]	595	300[d]	500	390[b,c]	400	300[b,c]
	(962)	(1611)	(388)	(1787)	(633)	(1470)	(709)
4	850[b,d]	1350	800[b,d]	id	800[c]	800	900[c]
	(1000)	(1950)	(650)		(1000)	(967)	(1011)
5	300[c,d]	300	300[c,d]	id	300[c]	300	300[c]
	(480)	(900)	(300)		(543)	(660)	(300)

d. Myelocytes (reference range: 0 cells/μL)

Day	All Cows	Cows with Bacteremia	Cows without Bacteremia	Non-Survivors	Survivors	Early Lactation	Mid/Late Lactation
1	0[a]	id	0[a]	0[a]	0[a]	0	0[a]
	(32)		(15)	(0)	(43)	(2092)	(15)
2	0[a]	100	0[a]	50	0[a]	0	0[a]
	(262)	(775)	(110)	(750)	(123)	(554)	(139)
3	100[b]	245	100[b]	250[b]	100[b]	100	100[b]
	(485)	(919)	(75)	(1117)	(233)	(627)	(414)
4	100	550†	0†	id	100	200	100
	(250)	(625)	(25)		(255)	(333)	(222)
5	0	0	0	id	0	0	0
	(88)	(625)	(33)		(100)	(150)	(25)

e. Lymphocytes (reference range: 2500 to 7500 cells/μL)

Day	All Cows	Cows with Bacteremia	Cows without Bacteremia	Non-Survivors	Survivors	Early Lactation	Mid/Late Lactation
1	1600	id	1300	1450	1700	2400	1150
	(2269)		(1424)	(1690)	(2475)	(2092)	(2350)
2	1900	2300	2400	3900	1700	2350	1800
	(2561)	(2614)	(2364)	(3348)	(2336)	(2449)	(2608)
3	1800	1750	2100	2550	1700	1700	2100
	(2794)	(3507)	(1988)	(4370)	(2163)	(3336)	(2523)
4	1700	1300	3200	id	1800	1600	1800
	(2167)	(1225)	(3250)		(2300)	(1800)	(2289)
5	1900	2100	1600	id	1700	1700	1600
	(2488)	(3067)	(2533)		(2529)	(1725)	(3250)

† Medians between compared groups (Cows with vs. without bacteremia; non-survivors vs. survivors; early vs. mid/late lactation) differ significantly ($P < .05$).
a,b,c,d. Medians within a group sharing common superscripts do not differ significantly ($P > .05$).
Medians without a superscript are not significantly different from any other median in that group.
id signifies that there were fewer than 3 cows in that group at that time point.

to .17), and the difference in band cell counts was significant on days 1 and 2 ($P < .05$). In contrast, cattle greater than 4 weeks into lactation followed the pattern outlined for all cows combined.

Discussion

Hematologic analysis of cows with acute coliform mastitis has the potential to yield clinically relevant information, because the leukogram frequently is abnormal,[2,4,21] and the abnormalities seen are rarely reported in cows with mastitis caused by gram-positive organisms.[24] However, there is little information on the value of this determination. Because of the relatively small number of study animals, heterogeneity of conditions inherent in a retrospective study of this nature, and analytical methods used, the statistical power to detect differences in this population was small. This low power coupled with the high level of confidence reinforces that the differences in specific cell counts represent true differences between populations of cows, and are likely to be clinically relevant. Additionally, this study provides a basis on which to build an understanding of hematologic changes in cattle with naturally occurring acute coliform mastitis.

Based on the data from this report, accurate interpretation of the leukogram requires knowledge of the duration of disease and stage of lactation of the cow. Panleukopenia was common, except in cows sick more than 3 days or in the first 4 weeks of lactation. Other

researchers report that neutropenia after experimental inoculation with either *E. coli* or endotoxin lasts less than the 3 days reported here.[1,3,9] This difference may be the result of natural infection or different methods of data analysis. Natural infections often last for several days,[2] while experimental infections frequently are cleared rapidly,[11] and endotoxin alone has a very short half-life.[16] Additionally, the median counts reported here were lower than the mean counts, which are reported by most authors. The higher mean counts often were the result of individual cows with relatively high cell counts (and usually in early lactation), and not representative of the group. Regardless of the reason, presence of neutropenia for up to 3 days cannot be taken as a sign of a poor prognosis for survival, and absence of neutropenia after 3 days or in cows in early lactation should not be taken as a sign of an incorrect diagnosis.

While resolution of leukopenia after several days of infection was expected, the infrequency of neutropenia in cows in early lactation with acute endotoxic disease was puzzling. Poor neutrophil recruitment to the mammary gland has been implicated as the cause of severe coliform mastitis in some early lactation cows,[12,23] and plasma corticosteroid concentrations remain high for most of the first month of lactation.[10] Corticosteroids are known inhibitors of neutrophil extravasation.[6] The lack of neutropenia in the face of acute coliform mastitis seen in most of these early lactation cows supports that there is inhibited margination and migration, possibly under the influence of corticosteroid, in the first weeks of lactation. Based on the low median neutrophil counts during the first three days of infection, this impaired diapedesis was not present in all early lactation cows.

Although daily changes in the leukogram from individual cows were not tested in this report, the changes in the pooled populations yielded little information of prognostic value, because non-surviving cows rarely lived to the fourth day of illness, when cell counts could be expected to rebound. Because worsening of the leukogram was difficult to interpret, both surviving and non-surviving cows had a strong initial leukopenic response and an insignificant decrease in mature neutrophil counts from day 1 to day 2. Non-surviving cows tended to have higher metamyelocyte and myelocyte counts after the peracute neutropenic phase on the second or third days of illness, suggesting that worsening might be seen as a shift to more immature forms, but these differences were not significant.

The greatest value of the leukogram in assessing cows with coliform mastitis may be in raising suspicion of bacteremia. Cows with bacteremia at admission had a delayed, less effective regenerative response, characterized by persistent neutropenia and high metamyelocyte and myelocyte counts. Differences in immature neutrophil forms were apparent as early as day 2, and therefore might be seen quickly enough to begin antibiotic treatment or obtain a blood sample for diagnostic bacteriologic culture. The duration of bacteremia is unknown, but it is likely that cattle with bacteremia had longer exposure to bacteria and endotoxin than cattle with infection confined to the mammary gland, and that these abnormalities in the leukogram are the result of this prolonged exposure. It is also possible that these effects contributed to the "worsening of CBC data" in non-surviving cows identified by others,[22] as it is logical that cows with bacteremia and not treated with antibiotics are less likely to survive the infection. These effects also provide a rational basis for the use of hematologic evaluation of cows suspected as having coliform mastitis. Physical examination has been demonstrated repeatedly to be a poor method of predicting the nature of the infecting organism with mastitis,[14,17,19] yet hematologic evaluation appears to be useful for this purpose.[12] These data also suggest that a single CBC obtained between days 2 and 4 of infection could be a useful, inexpensive way of identifying candidates for more vigorous diagnostic and therapeutic intervention. Managing cows with coliform mastitis without antibiotics has been advocated in recent years,[15,20] but such a herd treatment approach does not address the possibility of bacteremia in individual cows. Newer data that antibiotic use decreases severity[19] and that antibiotic treatment leads to similar survival percentages in cows with and without bacteremia,[2] support the judicious, if not routine, use of these medications. More data are needed to test the efficacy and economic benefit of this approach.

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Abstracts

Ultrasound-guided Percutaneous Lung Biopsy in Sheep

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Lung biopsies were taken through the ninth intercostal space on the two sides of the thorax of 10 clinically healthy sheep, using a Tru-Cut needle on one side and the Bard Biopty-System on the other. Nine of the 10 sheep remained clinically healthy, but one coughed transiently and had a mild bloody discharge from the right nostril immediately after the biopsy. The sheep were slaughtered 10 days later and the lungs and pleura were examined macroscopically; there were either no lesions or only small scars visible at the sites of the biopsies. However, well-developed subpleural nodules due to parasites were observed in some of the lungs. There were no adhesions between the costal and pulmonary pleurae. Of the 20 biopsy specimens, 18 were ideal for histological examination, and none of them was histologically normal. Mild interstitial pneumonia was diagnosed in 15 specimens, chronic bronchiolitis in nine specimens, and hyperplasia of smooth muscle or connective tissue in 17.

Johne's Disease in Sheep and Goats

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JOHNE's disease, or paratuberculosis, is a chronic enteric disease of ruminants and has a worldwide distribution. Economic losses from the disease can be measured in terms of reduced production as well as culled animals. Unlike cattle, in which the disease has been more extensively studied, chronic diarrhoea is not a consistent feature of Johne's disease in sheep and goats; the predominant clinical sign in these smaller ruminants is weight loss over a period of weeks or months. Johne's disease is caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis*. Sheep strains generally fail to grow, or grow only poorly, on routine culture media which support luxurious growth of the organism isolated from cattle. Goat strains tend to be more akin to cattle strains.