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Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin

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ABSTRACT

In recent years, the US dairy industry has experienced significant demographic changes, with an increase in the number of large herds. The objectives of the present study were to characterize clinical mastitis occurring in cows on large dairy herds in Wisconsin. Participating herds (n = 50) were required to have a minimum of 200 lactating animals, participate in monthly DHI testing (including monthly individual cow somatic cell count), use computerized herd records, use a milking routine that included fore-stripping quarters for detection of mastitis, and use antimicrobials to treat affected cows. After study personnel visited the farm, each herd was instructed to enroll the next 17 cows that experienced clinical mastitis, regardless of severity. At detection of clinical mastitis and 14 to 21 d after treatment ended, duplicate quarter milk samples were collected from all affected quarters and used for microbiological analysis. Treatments of affected cows were performed according to existing individual farm protocols. Cow level follow-up data was collected for 90 d after enrollment. Microbiological diagnoses at enrollment included gram-negative (35.6%), no growth (27.3%), gram-positive (27.5%), and other (9.6%). Of the 741 cases, the most prevalent pathogens were *Escherichia coli* (22.5%), followed by environmental streptococci (12.8%), *Klebsiella* spp. (6.9%), and coagulase-negative staphylococci (6.1%). Bacteriological cure was 75.0% for cases caused by gram-negative pathogens (n = 136), 50.8% for cases caused by gram-positive pathogens (n = 128), 47.5% for cases caused by other pathogens (n = 40), and 73.2% for cases which did not result in microbial growth (n = 123). Of the 583 cases with severity recorded, the distribution of mild, moderate, and severe symptoms was 47.8, 36.9, and 15.3%, respectively. The majority of cases presenting with severe symptoms were caused by gram-negative pathogens. Treatment cure was greater for gram-negative pathogens and cases for which no pathogens were recovered as compared with cases caused by other etiologies. Cows experiencing severe cases were more likely to receive multiple antimicrobial treatments.

Key words: large herd, clinical mastitis, characterization, severity score

INTRODUCTION

In recent years, the US dairy industry has experienced significant structural changes, with an increasing number of large herds responsible for a greater proportion of cow inventory and milk production as compared with small herds (USDA, 2009). Large herds differ from small herds in a variety of practices. Large herds have greater usage of computerized data records to track milk production, reproduction, and animal health as compared with small herds (USDA, 2009). Farmers with large herds also purchase more animals, are more likely to use diagnostic testing before purchase of animals, are more likely to vaccinate heifers, use more veterinary services, and have greater milk production per cow as compared with small herds (Hoe and Ruegg, 2006; USDA, 2007a). In addition, operators of large herds would be expected to observe more health problems in cattle due to the larger numbers of cows at risk for developing any health problem (USDA, 2007a).

Mastitis is the most prevalent health problem in dairy cows and one of the main reasons for permanently removing cows from herds (USDA, 2007b). Economic losses due to mastitis include reductions in milk production, increased cost of production, reduced milk quality, reduced longevity, increased labor and treatment costs, and transmission to other animals (Seegers et al., 2003; Gröhn et al., 2004; Pinzón-Sánchez and Ruegg, 2011). A variety of pathogens may cause mastitis in dairy cows; historically, the most common contagious mastitis pathogens have been *Streptococcus agalactiae* and *Staphylococcus aureus* (NMC, 1999). However, the adoption of modern milking practices has resulted in a considerable decline in the prevalence of these organisms in many modern US dairy herds (Makovec and Ruegg, 2003). Common environmental organisms include CNS, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Klebsiella* spp., and *Escherichia coli* (NMC, 1999). In the

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United States, several recent studies have shown that the most prevalent pathogens causing clinical mastitis in cows are usually organisms that originate from the environment (Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Schukken et al., 2011). Environmental mastitis pathogens are often associated with clinical mastitis, and few mastitis treatments have research that indicated efficacy against these organisms. Data that describe severity and treatment outcomes for clinical mastitis occurring on large, modern US dairy farms is sparse. The objective of this study was to characterize clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin.

MATERIALS AND METHODS

Herd and Cow Enrollment Criteria

Wisconsin dairy herds (n = 50) were recruited by extension agents and practicing veterinarians. Herds were required to have a minimum of 200 lactating animals, participate in monthly DHI testing (including monthly individual cow SCC), use computerized herd records, use a milking routine that included fore-stripping quarters for detection of mastitis, and use antimicrobials to treat affected cows. Extension agents (n = 18) and veterinarians (n = 2) were trained by the study personnel on data collection protocols. Additional herd-level management data was collected during the visit. At least one farm visit per evaluator (extension agents or veterinarians) was supervised by the study personnel. During the visit, farm workers were trained to classify severity of clinical mastitis using a previously defined scoring system (Pinzón-Sánchez and Ruegg, 2011): mild (grade 1) when only the milk was abnormal; moderate (grade 2) when abnormal milk was accompanied by swelling or redness of mammary gland; or severe (grade 3) when the cow exhibited systemic signs of illness such as depression, anorexia, dehydration, or fever. After the farm visit, farmers were instructed to enroll the next 17 cows that experienced clinical mastitis. Each cow was eligible for enrollment only once. Sample size was estimated based on the expected distribution of mastitis pathogens.

Sampling and Data Collection

Cases were detected by trained farm personnel who collected duplicate quarter milk samples from only the clinically affected quarter(s) before treatment (**PRE**). After collection, cows were treated according to individual farm protocol. Farm personnel collected a second set of duplicate quarter milk samples from the enrolled quarter(s) approximately 14 to 21 d after the end of treatment (**POST**). Samples were frozen and mailed to University of Wisconsin-Madison's milk quality laboratory.

Farm personnel recorded data for each case including information about cow characteristics, the date the clinical mastitis case was detected, affected quarter(s), severity grade, drugs and doses used for treatment, number of days treated with each drug, the date milk returned to normal appearance (clinical cure), and the date milk was returned to the bulk tank. After enrollment, if a cow experienced an occurrence of a new clinical case in any quarter within 90 d, another set of duplicate quarter samples were collected before treatment from the affected quarter(s), frozen, and mailed to the laboratory. For repeated cases, study personnel collected the same data as described above for a PRE milk sample. Paperwork was left on the farm to collect information about events that occurred within 90 d after enrollment. Farmers were instructed to record information about removal (death or culling) of an enrolled cow from the herd, reason and date the cow was removed, date of the end of lactation (dry cows), if a cow lost a quarter (dried off naturally or therapeutically), any disease (such as pneumonia or foot problems), as well as drugs and doses used for treatment. The data from the forms were cross-checked with information from on-farm record-keeping systems. Milk production and SCC for each cow were obtained from the DHI monthly test occurring 14 to 52 d after treatment ended. Additional information collected included previous cases of clinical mastitis in the current lactation, quarter(s) affected, and drugs and doses used for treatment. Milk production and SCC before the clinical mastitis case for each cow were obtained from the DHI monthly test occurring 3 to 34 d before occurrence of the enrolled clinical mastitis case.

Microbiological Analysis

Upon arrival at the laboratory, all frozen samples were thawed at room temperature and 100 μL of milk from each duplicate sample were plated onto each half of a blood agar and 10 μL were plated onto a quarter of a MacConkey agar. Plates were incubated at 37°C for 24 to 48 h. Microbiologic procedures were conducted according to guidelines (NMC, 1999). *Staphylococcus aureus* were differentiated from other staphylococci by means of mannitol and tube coagulase reactions. Suspected *Streptococcus* spp. were identified as catalase negative, gram-positive cocci by the Christie, Atkins, Munch-Petersen test and esculin reaction. Gram-negative bacteria were identified using MacConkey agar, Gram stain, motility, indole, ornithine reactions, oxidase, and growth on triple sugar iron slants.

Infection status was defined at the quarter level. An IMI was defined as the presence of 300 cfu/mL of identical colonies based on microbiologic procedures described above. Mixed infection was defined as the recovery of at least 300 cfu/mL of 2 different types of bacteria from a sample. Milk samples were considered contaminated if 3 or more dissimilar colony types were found in the same sample. Criteria used to define etiology of quarter cases based on microbiological results from duplicate milk samples were based on Pinzón-Sánchez and Ruegg (2011). Etiologies were defined as (1) results were identical from both duplicate milk samples; (2) no bacteria were recovered from 1 sample but pathogen was recovered from the other sample; (3) 1 sample was contaminated and pathogen was recovered from the other sample; (4) 1 sample was contaminated and no bacteria was recovered from the other sample; or (5) 1 sample was missing but pathogen or no bacteria was recovered from the duplicate.

Statistical Analysis

Definitions. Case definition for symptoms of clinical mastitis was farm-specific. Most farms mainly use abnormal milk every milking and swollen quarters to recognize clinical mastitis. Cows with $\geq$2 quarters affected at the same time were excluded because they were a relatively small proportion of overall cases and analysis would have been complicated relative to pathogen effects and the effect of the case on DHI values for SCC and milk yield.

Treatment cure was assessed by comparing microbiological results from both samples PRE and POST detection of clinical mastitis. Bacteriological cure was defined when a pathogen was identified on both PRE milk samples but both POST milk samples from the same quarter was culture negative. Self cure was defined when no pathogens were recovered from both PRE and POST milk samples. Enrolled quarters classified as bacteriological cure and self cure were together categorized as treatment cure. Bacteriological failure was defined when the same pathogen were recovered from PRE and POST milk samples, in either pure or mixed infection. New IMI were identified when a pathogen not present in 1 of the 2 PRE milk samples was recovered from 1 of the 2 POST milk samples. Enrolled quarters classified as bacteriological failure and new IMI were categorized together as not experiencing treatment cure. Somatic cell count reduction after infection was defined at the cow level as an SCC below 200,000 cells/mL at the DHI test day occurring between 14 to 52 d post-treatment (Pinzón-Sánchez and Ruegg, 2011). Milk production deviation was defined at the cow level as the difference between milk production (kg) at DHI monthly test occurring between 3 to 34 d before occurrence of the clinical mastitis case and milk production (kg) at the DHI monthly test occurring between 14 to 52 d post-treatment. Recurrence of clinical mastitis during the 90-d follow-up period was defined as the occurrence of a new clinical mastitis case in any quarter of the same cow after the end of treatment period for the enrolled case and within at least 14 d after enrollment. Culling was defined as cows leaving the herd during the 90-d follow-up period because of sale or death, as opposed to remaining in the herd (lactating or dry).

Statistical Procedures. Statistical analyses were carried out using SAS version 9.3 (SAS Institute, 2011). Descriptive statistics were used to verify data accuracy, detect missing data, and observe frequency distributions. The distribution of continuous variables was analyzed using frequency histograms. Summary statistics were produced using measures of central tendency (mean, median). For categorical variables, distribution was analyzed using frequency tables. The PROC FREQ function (SAS Institute, 2011) was used to perform all chi-squared analyses. Chi-squared analyses were used to determine if each explanatory variable with a categorical distribution was independent of severity scores. The PROC GLM function (SAS Institute, 2011) was used to perform ANOVA tests to determine if each continuous distribution was independent of severity scores.

Differences in DIM at enrollment of first cases of mastitis in the current lactation were determined for the most prevalent etiologies (*E. coli*, *Klebsiella* spp., environmental streptococci, CNS, *Staph. aureus*, and no growth) and were evaluated using PROC LIFETEST (SAS Institute, 2011). In addition, differences in DIM at enrollment for first cases of mastitis among mild, moderate, and severe cases were evaluated using PROC LIFETEST (SAS Institute, 2011). Days in milk at enrollment of first cases was used as time in the survival analysis.

RESULTS

Herd Characteristics

Of approximately 1,100 herds in Wisconsin (USDA, 2009) that met the herd size criteria, 50 herds were enrolled. Herd size ranged from 170 to 2,728 lactating cows, and mean daily milk production per cow was 33.5 kg (range = 21.0–40.8 kg). The average bulk tank SCC was 219,000 cells/mL (range = 87,000–432,000 cells/mL). In all herds, cows were housed in freestalls and sand was the most common bedding source for lactating cows (n = 20). The other types of bedding used were sawdust, mattresses, a combination of mattresses and sawdust, or a combination of sand and sawdust.

On most farms, cows were milked in parallel parlors. All cows were milked using a complete milking routine consisting of stripping of foremilk, pre- and postdipping disinfection, and drying of teats. Antimicrobial dry cow therapy was used to treat all quarters of all cows in all herds; internal teat sealant was used on 42 herds and 8 herds used an external sealant at drying off. A total of 38 herds (76.0%) had written protocols for mastitis treatments. Mastitis treatments were performed by an average of 3.4 people per herd. Use of gram-negative core-antigen coliform vaccination (2–4 times during the lactation) as part of herd health program was reported by 22 herds (44%). Of enrolled herds, 11 (22%) indicated that they cultured all cases of clinical mastitis and 25 (50%) indicated that they cultured only selected cases as the routine mastitis management. The minimum and maximum number of cases enrolled in the study per farm was 6 and 29, respectively; 27 herds enrolled the suggested 17 cows.

Microbiological Results

A total of 832 cows were initially enrolled. Among all enrolled cows, 91 were excluded for having ≥2 quarters affected at the same time (n = 45; 5.4%) and PRE milk samples contaminated (n = 46; 5.5%). Consequently, data from 741 cows from 50 herds were retained in the study. Microbiological diagnosis of the PRE samples was distributed as gram-negative (35.6%), no growth (27.3%), gram-positive (27.5%), and other (9.6%; Table 1). The most prevalent pathogens isolated from PRE samples were *E. coli* (22.5%), followed by environmental streptococci (12.8%), *Klebsiella* spp. (6.9%), and CNS (6.1%; Table 1). *Escherichia coli* and environmental streptococci were isolated from milk samples originating from 42 herds and 38 herds, respectively. *Staphylococcus aureus* (2.8%) were not a frequent cause of clinical mastitis, but were isolated from 14 herds (Table 1). The number of cases with usable POST milk samples was decreased to 427 because milk samples were not collected (n = 227) or were contaminated (n = 87). The most prevalent pathogens recovered from POST milk samples were environmental streptococci and CNS (Table 1).

The overall proportion of treatment cure was 64.6% (n = 276/427). Treatment cure was greatest for cases with no growth on the PRE sample (73.2%) and for cases caused by gram-negative bacteria (75.0%) and was less for cases caused by gram-positive pathogens (50.8%) or cases caused by other pathogens (47.5%; $P < 0.001$; Table 2). The proportions of treatment cure were 75.6 and 78.3% for cases caused by *E. coli* and *Klebsiella* spp., respectively (Table 2). The proportions of treatment cure were 52.8 and 55.9% for cases caused

Table 1. Microbiological diagnosis of milk samples obtained from clinical mastitis cases (n = 741) occurring in cows on Wisconsin dairy herds (n = 50) collected at enrollment (PRE) and 14 to 21 d after the end of treatment (POST)

	PRE			POST		
Microbiological diagnosis	n	Percentage	Number of herds	n	Percentage	Number of herds
Total gram negative	264	35.6	47	31	4.2	25
Escherichia coli	167	22.5	42	12	1.6	11
Klebsiella spp.	51	6.9	22	4	0.5	4
Enterobacter spp.	27	3.6	18	5	0.7	5
Other gram negative[1]	19	2.6	13	10	1.3	8
Total gram positive	204	27.5	48	99	13.4	41
Environmental streptococci	95	12.8	38	23	3.1	16
Coagulase-negative staphylococci	45	6.1	28	29	3.9	22
Staphylococcus aureus	21	2.8	14	15	2.0	11
Enterococcus spp.	15	2.0	11	4	0.5	3
Trueperella pyogenes	15	2.0	13	3	0.4	3
Gram-positive *Bacillus* spp.	10	1.3	9	13	1.7	7
Corynebacterium spp.	—	—	—	8	1.1	7
Other gram positive[2]	3	0.4	3	4	0.5	4
Total other pathogens	71	9.6	37	21	2.8	15
Yeast	23	3.1	15	5	0.7	4
Mixed infection	48	6.5	29	16	2.2	13
No growth	202	27.3	49	276	37.2	44
Contaminated samples[3]	—	—	—	87	11.7	34
Missing samples	—	—	—	227	30.6	48
Total	741	100.0	50	741	100.0	50

[1]Gram-negative bacilli, gram-negative lactose-negative rods, *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., and *Serratia* spp. were coded as other gram negative.

[2]Actinomycete, *Lactococcus* spp., and *Lactobacillus* spp. were coded as other gram positive.

[3]Milk samples that were contaminated at enrollment were excluded (n = 46).

Table 2. Proportion of selected outcomes in a 90-d follow-up period for clinical mastitis cases (n = 741) occurring in cows on Wisconsin dairy herds (n = 50) by microbiological diagnosis at enrollment

	Treatment cure		SCC reduction[1]		Recurrence within 90 d		Removed from herd within 90 d	
Diagnosis at enrollment	n (%)	Total	n (%)	Total	n (%)	Total	n (%)	Total
Gram negative combined	102 (75.0)[a]	136	88 (57.5)[a]	153	45 (31.2)[a]	144	63 (26.7)[a]	236
Escherichia coli	65 (75.6)	86	64 (57.1)	112	25 (26.9)	93	35 (23.3)	150
Klebsiella spp.	18 (78.3)	23	8 (50.0)	16	10 (40.0)	25	17 (38.6)	44
Other gram negative[2]	19 (70.4)	27	16 (64.0)	25	10 (38.5)	26	11 (26.2)	42
Gram positive combined	65 (50.8)[b]	128	70 (52.2)[a]	134	27 (20.6)[b]	131	26 (14.6)[b]	178
Environmental streptococci	28 (52.8)	53	37 (59.7)	62	10 (18.5)	54	10 (11.9)	84
CNS	19 (55.9)	34	13 (46.4)	28	9 (25.7)	35	5 (21.2)	41
Other gram positive[3]	18 (43.9)	41	20 (45.5)	44	8 (19.0)	42	11 (20.7)	53
Other[4]	19 (47.5)[c]	40	26 (53.1)[a]	49	7 (15.9)[b]	44	7 (10.9)[b]	64
No growth	90 (73.2)[a]	123	75 (59.5)[a]	126	19 (13.7)[b]	139	24 (13.1)[b]	183
Total[5]	276 (64.6)	427	259 (56.1)	462	98 (21.4)	458	120 (18.1)	661

[a–c]Means within a column with the same superscript are not significantly different ($P < 0.05$; categories: gram negative, gram positive, other, or no growth).

[1]Somatic cell count below 200,000 cells/mL from the DHI monthly test occurring from 14 to 52 d after end of treatment.

[2]*Enterobacter* spp., gram-negative bacilli, gram-negative lactose-negative rods, *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., and *Serratia* spp. were coded as other gram negative.

[3]*Staphylococcus aureus*, *Enterococcus* spp., *Trueperella pyogenes*, gram-positive *Bacillus* spp., *Corynebacterium* spp., actinomycete, *Lactococcus* spp., and *Lactobacillus* spp. were coded as other gram positive.

[4]Infections caused by yeast or mixed infection (the recovered of at least 3 colonies of 2 different types of bacteria from a sample).

[5]Based on the data available for each outcome (data available for bacteriological cure = 427; SCC reduction = 465; recurrence within 90 d = 463; and removed from herd within 90 d = 667).

by environmental streptococci and CNS, respectively (Table 2). The treatment cure for cases caused by other gram-positive pathogens was 43.9% (Table 2).

The overall SCC reduction after infection was 56.1% (n = 259/462) and did not differ based on etiology ($P = 0.08$). The proportion of cows that experienced SCC reduction after infection was 57.5% for cases caused by gram-negative pathogens (n = 153), 52.2% for cases caused by gram-positive pathogens (n = 134), 53.1% for cases caused by other pathogens (n = 49), and 59.5% for cases which did not result in microbial growth (n = 126; Table 2).

The overall recurrence of another case of clinical mastitis within the 90-d follow-up period was 21.4% (n = 98/458) and was greater for cows that experienced cases caused by gram-negative pathogens at enrollment compared with other etiologies (Table 2). Of cows with recurrent cases, 44.9% occurred in the same quarter, 36.7% in a different quarter, 14.3% in multiple quarters, and for 4% of cows this information was not available. The mean number of days until recurrence was 47.2 (from 15–86 d). The proportion of recurrence was 31.2% for cases caused by gram-negative pathogens (n = 144), 20.6% for cases caused by gram-positive pathogens (n = 131), 15.9% for cases caused by other pathogens (n = 44), and 13.7% for cases which did not result in microbial growth (n = 139; Table 2). The proportion of recurrence was 26.9 and 40.0% for cases caused by *E. coli* and *Klebsiella* spp., respectively (Table 2). The proportion of recurrence was 18.5 and 25.7% for cases caused by environmental streptococci and CNS, respectively (Table 2).

The overall removal from the herd within 90 d of enrollment in the study was 18.1% (n = 120/661) and was greater for cows with cases of clinical mastitis caused by gram-negative pathogens ($P < 0.001$; Table 2). The average number of days after the occurrence of the enrolled clinical case until removal was 36.3. The proportion of cows that were removed from herds was 26.7% for cases caused by gram-negative pathogens (n = 63/236), 14.6% for cases caused by gram-positive pathogens (n = 26/178), 10.9% for cases caused by other pathogens (n = 7/64), and 13.1% for cases which did not result in microbial growth (n = 24/183; Table 2). The proportion of cows that were removed from herds within 90 d was 23.3 and 38.6% for cows affected by cases caused by *E. coli* and *Klebsiella* spp., respectively (Table 2). The proportion of cows that were removed from herds within 90 d was 11.9 and 21.2% for cows affected by cases caused by environmental streptococci and CNS, respectively (Table 2).

For first cases of clinical mastitis occurring in the enrolled lactation (n = 392/741), etiology was not associated with DIM at occurrence ($P = 0.17$; Figure 1). With the exception of cases caused by *Staph. aureus* or cases that resulted in no growth, approximately 30% of first cases of clinical mastitis occurred within 60 d after calving (Figure 1).

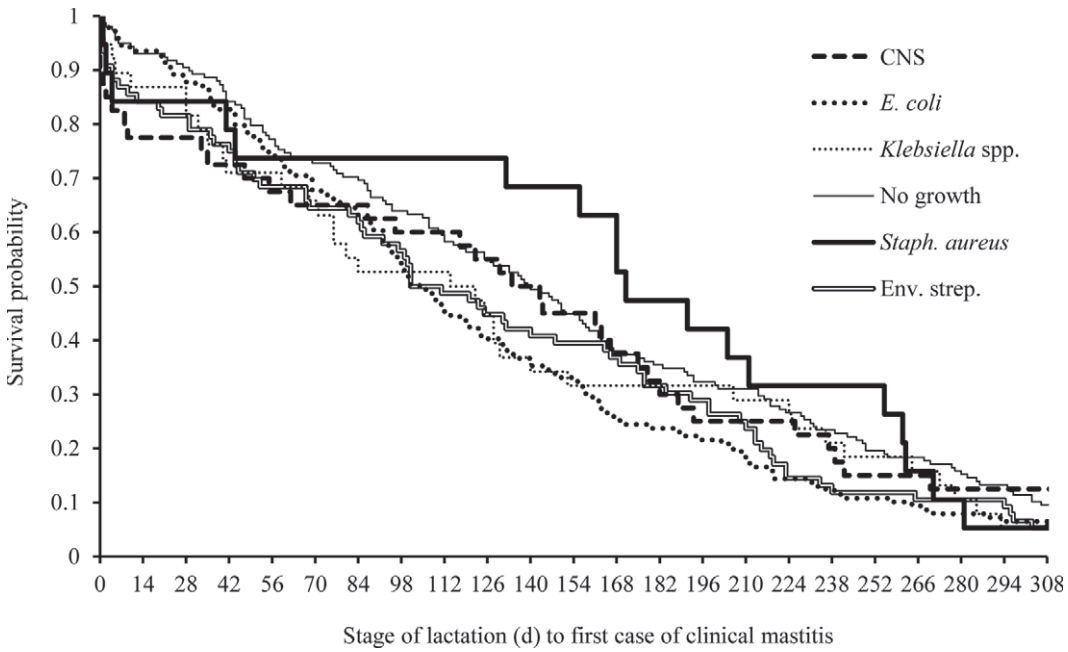

Figure 1. Survival graph representing the probability of the first case of clinical mastitis in the current lactation by stage of lactation stratified by etiology of milk sample at enrollment. The main microbiological diagnoses included were CNS (n = 38), *Escherichia coli* (*E. coli*; n = 109), *Klebsiella* spp. (n = 35), no growth (n = 128), *Staphylococcus aureus* (*Staph. aureus*; n = 19), and environmental streptococci (Env. strep.; n = 63). Statistical significance was observed at $P = 0.17$.

Characterization of Clinical Mastitis Cases by Severity

Severity scores were recorded for 583 cases (78.7%) at enrollment. The distribution of clinical mastitis cases with mild, moderate, and severe symptoms was 47.8, 36.9, and 15.3%, respectively (Table 3). Microbiological diagnosis at the PRE milk sample was associated with severity ($P < 0.001$; Table 4). Of cows exhibiting mild symptoms, 33.3% of cases were caused by gram-positive pathogens and 34.1% were cases which did not result in microbial growth in contrast to lesser proportions of cases caused by gram-negative or other pathogens (Table 4). Most of the cases presenting with moderate symptoms were caused by gram-negative pathogens (33.5% of all moderate cases) and gram-positive pathogens (29.3% of all moderate cases). In contrast, the majority of cases presenting with severe symptoms were caused by gram-negative pathogens (75.3%; Table 4). The proportion of severity scores was similar for *E. coli* and *Klebsiella* spp. (Table 3). Very few cases of mastitis caused by gram-positive pathogens (including environmental streptococci and CNS) exhibited severe symptoms.

Parity was not associated with the severity score of clinical mastitis cases ($P = 0.16$; Table 4). The distribution of cases by parity was 18.9% (n = 119) for first lactation, 29.9% (n = 173) for second lactation, 25.1% (n = 145) for third lactation, and 26.1% (n = 151) for fourth or greater lactation (Table 4). Parity information was not available for 5 cows. History of previous clinical mastitis was not associated with the severity score of clinical mastitis cases ($P = 0.48$; Table 4). Only 17.1% of cows had a history of at least 1 previous case of clinical mastitis within the studied lactation. Among cows with a history of clinical mastitis within the studied lactation (n = 100), 17.9, 14.9, and 20.2% of the enrolled cases were presenting mild, moderate, and severe symptoms, respectively.

Of the 558 cows that had information about severity scores and were treated with antimicrobials, 376 (67.4%) were treated solely with a single antimicrobial administered using either an intramammary or systemic route. In addition, 182 of 558 (32.6%) received multiple antimicrobial treatments (concomitant intramammary and systemic or an additional antimicrobial following the primary treatment). A single antimicrobial was used for treatment of 82.4 and 69.0% of cases presenting mild and moderate symptoms, respectively. In contrast, almost 79.8% of cows scored as having severe symptoms received multiple antimicrobial treatments. Cows experiencing severe symptoms were 12.4 times more likely to receive multiple antimicrobials compared with cows experiencing mild and moderate symptoms (Table 4).

Overall, treatment cure was 63.1% (n = 229) and increased with severity score ($P = 0.04$; Table 4). Treatment cures were 56.2, 67.4, and 79.5% for cows

Table 3. Distribution of etiology of milk samples from clinical mastitis cases occurring in cows (n = 583) on Wisconsin dairy herds (n = 50) by severity score (n = 583)

	Severity score[1]							
	Mild (1)		Moderate (2)		Severe (3)		Total	
Microbiological diagnosis	n	%	n	%	n	%	n	%
Escherichia coli	35	27.8	48	38.1	43	34.1	126	21.6
Klebsiella spp.	13	30.2	16	37.2	14	32.6	43	7.4
Other gram negative[2]	16	47.1	8	23.5	10	29.4	34	5.8
Environmental streptococci	39	60.9	24	37.5	1	1.6	64	11.0
CNS	25	61.0	15	36.6	1	2.4	41	7.0
Staphylococcus aureus	10	52.6	9	47.4	0	0.0	19	3.3
Other gram positive[3]	19	52.8	15	41.7	2	5.5	36	6.2
Yeast	3	20.0	9	60.0	3	20.0	15	2.6
Mixed	24	58.6	16	39.0	1	2.4	41	7.0
No growth	95	57.9	55	33.6	14	8.5	164	28.1
Total	279	47.8	215	36.9	89	15.3	583	100.0

[1]Mild (grade 1) when only the milk was abnormal; moderate (grade 2) when abnormal milk was accompanied by swelling or redness of mammary gland; or severe cases (grade 3) when cow exhibited systemic signs of illness such as depression, anorexia, dehydration, or fever.

[2]*Enterobacter* spp., gram-negative bacilli, gram-negative lactose-negative rods, *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., and *Serratia* spp. were coded as other gram negative.

[3]*Enterococcus* spp., *Trueperella pyogenes*, gram-positive *Bacillus* spp., *Corynebacterium* spp., actinomycete, *Lactococcus* spp., and *Lactobacillus* spp. were coded as other gram positive.

experiencing mild, moderate, and severe symptoms of clinical mastitis cases, respectively. Cows that experienced severe symptoms of mastitis were 2.5 times more likely to exhibit treatment cure compared with cows experiencing mild and moderate symptoms (Table 4). The proportion of cows that experienced SCC reduction at the DHI test date 14 to 52 d after the case was detected was 54.7% and was not associated with severity score ($P = 0.97$; Table 4). Recurrence of clinical mastitis was not associated with severity ($P = 0.41$; Table 4). Within the 90-d follow-up period, 21.4% of enrolled cows had a recurrence.

Removal from herd was associated with severity ($P = 0.04$; Table 4). Within the 90-d follow-up period, 18.2% of enrolled cows were removed from herds. Cows exhibiting severe cases were 2.9 times more likely to be removed from the herd compared with cows experiencing mild and moderate symptoms (Table 4). The average DIM for removing cows from herds was 147 d and they were removed an average of 34.3 d after the occurrence of the enrolled case. Reasons reported for removing cows from herds included mastitis (n = 48), lameness (n = 6), reproduction and mastitis (n = 5), reproduction (n = 4), other reasons (n = 19), and unknown (n = 13). Of cows with mild cases of clinical mastitis, 13.5% were removed in contrast to 18.9 and 30.9% of cows with moderate and severe cases, respectively.

The median DIM of cows at enrollment cases was 133 d and did not differ based on severity score of clinical mastitis ($P = 0.82$; Table 5). Likewise, for the subset of enrolled cases that were the first case of mastitis for the cow within the lactation, DIM did not differ based on the severity score of clinical mastitis ($P = 0.73$; Figure 2). The average $\log_{10}$SCC at the DHI test previous to the case for all cows was 5.2 and $\log_{10}$SCC was less for cows that experienced moderate and severe cases (4.9) as compared with mild cases ($P = 0.001$; Table 5). Milk production at the DHI test 2 to 32 d before the enrolled clinical mastitis case was 45 kg/cow per day and was greater for cows that experienced severe cases compared with production of cows that experienced mild and moderate cases ($P = 0.05$; Table 5). Milk production at the DHI test 14 to 52 d after enrolling was 40 kg/cow per day and did not differ based on severity scores ($P = 0.11$; Table 5). Milk deviation (difference in milk production at the DHI test 2 to 32 d before the enrolled case and milk production at the DHI test 14 to 52 d after the enrolled case) was −5.1 kg/cow per day and was greatest for cows that experienced severe cases of mastitis as compared with mild and moderate cases ($P = 0.001$; Table 5).

DISCUSSION

In recent years, the US dairy industry has experienced significant structural changes, with an increasing number of large herds responsible for a greater proportion of cow inventory and milk production compared with smaller herds (USDA, 2009). The population of herds included in this study (average herd size = 775 milking cows) represents a significant proportion of milk produced in Wisconsin. Including a larger number of herds in this study is advantageous, as compared with

Table 4. Characteristics of clinical mastitis cases (n = 583) occurring in cows on Wisconsin dairy herds (n = 50) by severity

			Severity of clinical mastitis case[1]							
	Overall		Mild (1)		Moderate (2)		Severe (3)			Severe compared with mild and moderate cases[2]
Category	n	%	n	%	n	%	n	%	*P*-value	Odds ratio	CI
Diagnosis[3]									<0.001	8.0	4.7–13.5
GP	160	27.4	93	33.3	63	29.3	4	4.5			
GN	203	34.8	65	22.9	72	33.5	67	75.3			
NO	164	28.1	96	34.1	55	25.6	14	15.7			
OT	56	9.6	29	9.7	25	11.6	4	4.5			
Parity[4]									0.16		
1	119	18.9	55	20.0	39	18.2	15	16.9			
2	173	29.9	75	27.5	71	33.2	27	30.3			
3	145	25.1	64	23.2	50	23.4	31	34.8			
>3	151	26.1	81	29.3	54	25.2	16	18.0			
History CM[5]									0.48		
No	483	82.9	229	82.1	183	85.1	71	79.8			
Yes	100	17.1	50	17.9	32	14.9	18	20.2			
Drug[6]									<0.001		
Single	376	67.4	211	82.4	147	69.0	18	20.2			
Multi	182	32.6	45	17.6	66	31.0	71	79.8		12.4	7.1–21.7
TC[7]									0.04		
No	134	36.9	82	43.8	43	32.6	9	20.5			
Yes	229	63.1	105	56.2	89	67.4	35	79.5		2.5	1.2–5.4
SCCR[8]									0.97		
No	160	45.3	79	45.7	62	44.6	19	46.3			
Yes	193	54.7	94	54.3	77	55.4	22	53.7			
Recurrence[9]									0.41		
No	360	78.6	185	80.4	128	75.3	47	81.0			
Yes	98	21.4	45	19.6	42	24.7	11	19.0			
Removed [10]									0.04		
No	427	81.8	217	86.6	154	81.1	56	69.1			
Yes	95	18.2	34	13.5	36	18.9	25	30.9		2.9	1.4–3.8

[1]Mild (grade 1) when only the milk was abnormal; moderate (grade 2) when abnormal milk was accompanied by swelling or redness of mammary gland; or severe cases (grade 3) when cow exhibited systemic signs of illness such as depression, anorexia, dehydration, or fever.

[2]Calculated only for outcomes where a significant association was detected; comparison is odds of severe cases compared with mild and moderate cases (95% confidence interval).

[3]GP = gram-positive pathogens; GN = gram-negative pathogens; NO = no growth; OT = others. Gram negative was the reference category.

[4]Data were not available for 5 cows.

[5]History CM = other clinical mastitis cases in the current lactation.

[6]Cows treated with a single antimicrobial or multiple antimicrobials (either intramammary or systemic).

[7]TC = treatment cure. Data were available for 363 cows.

[8]SCCR = somatic cell count reduction after infection was defined at the cow level as SCC below 200,000 cells/mL at the DHI test day occurring between 14 to 52 d post-treatment of clinical mastitis case. Data were available for 353 cows.

[9]Cows that exhibited recurrent case of clinical mastitis in any quarter. Cows were excluded if they were removed from the herd or dried before completing the 90-d follow-up period or experiencing a recurrence. Data were available for 458 cows.

[10]Cows removed from herd within the 90-d follow-up period. Cows excluded if they were dried before completing the 90-d follow-up period. Data were available for 522 cows.

studies where a single commercial or experimental herd was used, because a variety of management practices and pathogens causing mastitis were included. Much of the previous information about clinical mastitis occurring in US dairy herds is based on data that that has been collected from smaller herds. The purpose of this study was to describe and characterize mastitis occurring on larger dairy farm that milk cows using modern technologies.

Most cases of clinical mastitis included in this study were caused by gram-negative pathogens, followed by gram-positive pathogens and cases where no pathogens were recovered. The common pathogens recovered were *E. coli*, environmental streptococci, *Klebsiella* spp., and CNS. The distribution of pathogens observed in this study is similar to other studies that included milk samples from clinical mastitis cases from modern US dairy farms (Hogan et al., 1989; Makovec and Ruegg, 2003; Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Schukken et al., 2011). However, the distribution of pathogens differs from studies conducted using smaller herds characteristic of Canada and the Netherlands, where *Staph. aureus* was the most commonly pathogen isolated from clinical mastitis cases (Barkema

Table 5. Characteristics of clinical mastitis cases (n = 583) occurring in cows on Wisconsin dairy herds (n = 50) by severity score

	Severity of clinical mastitis case												
	Mild (1)			Moderate (2)			Severe (3)			Overall			
Characteristic	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	*P*-value
SCC (log_{10}) before enrollment[1]	164	5.3[a]	0.8	142	5.1[b]	0.8	47	4.9[b]	0.5	353	5.2	0.7	0.001
Milk production before enrollment[2]	167	44.6[a]	11.8	142	44.3[a]	10.9	47	48.8[b]	10.6	356	45.0	11.4	0.05
Postmilk after treatment[3]	174	41.4[a]	10.2	139	39.4[a]	11.3	41	38.2[a]	11.6	354	40.3	10.9	0.11
Milk deviation[4]	141	−3.7[a]	8.9	106	−5.1[a]	11.3	31	−11.2[b]	11.2	278	−5.1	10.3	0.001
DIM[5]	279	136.0[a]	—	215	136.0[a]	—	89	125.0[a]	—	583	133.0	—	0.82

[a,b]Means within a row with the same superscript are not significantly different ($P < 0.05$).

[1]Data were not available for all 583 cows.

[2]Production in kilograms per cow per day. Value from monthly DHI test 2 to 32 d previous to the enrolled case.

[3]Milk production (kg/cow per day) after enrollment at cow level was obtained at DHI monthly test occurring from 14 to 52 d after end of treatment.

[4]Difference in milk production (kg/cow per day) before and after enrollment case.

[5]Days in milk was continuous, non-normal distribution.

et al., 1998; Olde Riekerink et al., 2008). Barkema et al. (1998) reported that herds with low bulk tank SCC had a higher incidence of clinical mastitis caused by *E. coli* and *Strep. dysgalactiae*, and herds with a high bulk tank SCC had a higher incidence of clinical mastitis caused by contagious mastitis pathogens such as *Staph. aureus*. In a Canadian study, cows kept in tiestall barns had proportionally more clinical *Staph. aureus* and *Strep. uberis* mastitis compared with those in freestall barns (Olde Riekerink et al., 2008). In the present study, the average bulk tank SCC was 219,000 cells/mL and all cows were housed in freestall barns. In the present study, *Staph. aureus* was recovered from a limited number of cows and herds and *Strep. agalactiae* was not recovered from any cases. This suggests that progress in controlling contagious pathogens has occurred, as previously described (Makovec and Ruegg, 2003; Hillerton and Berry, 2005; LeBlanc et al., 2006).

Escherichia coli, environmental streptococci, *Klebsiella* spp., and CNS are considered environmental pathogens. Their primary source is considered to be the cow's environment, which includes bedding materials,

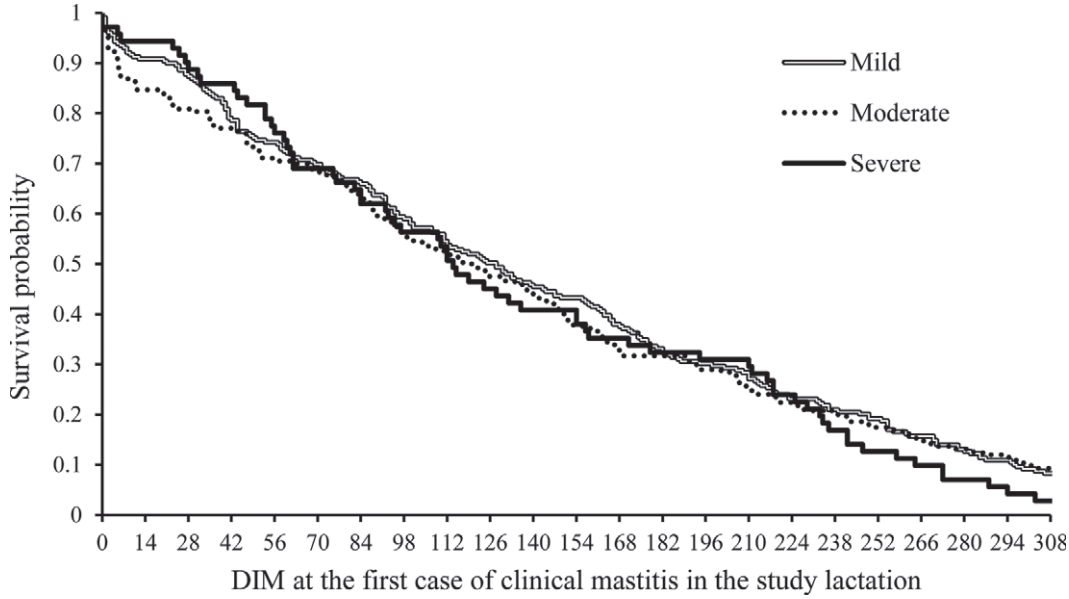

Figure 2. Survival graph representing the probability of the clinical mastitis case for the first time in the current lactation by stage of lactation stratified by severity (mild = 166 cases; moderate = 138 cases; severe = 63 cases). Statistical significance was observed at $P = 0.67$.

soil, and manure (Smith and Hogan, 1993). *Escherichia coli* were isolated from most herds in the present study and represented approximately twice as many cases as the next most prevalent pathogen. This finding demonstrates the importance of this pathogen in large dairy herds. *Escherichia coli* are considered an opportunistic pathogen and different factors have been shown to be associated with IMI caused by this microorganism; these include high daily milk yield, leaking milk, teat lesions, a reduced capacity of the immune system, exposure in an environmental source such as bedding material and dirt, management practices in the milking parlor, and host defense mechanisms (Schukken et al., 1991; Barkema et al., 1999; Burvenich et al., 2003; Hogan and Smith, 2003; Ericsson et al., 2009).

Klebsiella spp. (another gram-negative pathogen), was the third most frequently isolated pathogen. *Klebsiella* spp. may cause either individual clinical mastitis cases or outbreaks in dairy herds (Munoz et al., 2007; Olde Riekerink et al., 2008). Economic losses due to *Klebsiella* spp. are much greater when compared with losses due to *E. coli* because of reduced survival and milk production (Erskine et al., 2002; Gröhn et al., 2004, 2005). In the present study, 39% of cows that had clinical mastitis cases caused by *Klebsiella* spp. were removed from herds within 90 d, whereas only 23% of cows that had cases caused by *E. coli* were removed from herds. The number of cows that had clinical mastitis caused by *Klebsiella* spp. removed from the herds could influence the results of treatment cure, as cows that presented clinical cure were more likely to be kept in the herd. As these cows were removed from the herds before POST milk samples were collected, they were not included in the calculations. Decreased milk production is an important reason that cows are often culled (Gröhn et al., 2005; Pinzón-Sánchez and Ruegg, 2011). Other researchers demonstrated that severity and chronicity of clinical mastitis result in production losses as large as 5 to 6 kg/d for heifers and 10 kg/d for cows considering 3,071 cows from 2 New York State farms (Gröhn et al., 2004). In the present study, the greatest milk deviation (difference in milk production before and after enrollment case) was experienced for cows that exhibited severe symptoms.

Environmental streptococci were the most common gram-positive pathogen responsible for clinical mastitis in this study; they were also frequently isolated in cases of clinical mastitis in other studies performed in the United States and Canada (Olde Riekerink et al., 2008; Schukken et al., 2009). Furthermore, CNS were isolated from cows from more than half of herds included in this study. Coagulase-negative staphylococci are part of the normal flora of the teat skin, often colonize the streak canal, and have traditionally been considered opportunistic pathogens (Aarestrup et al., 1999; Pyörälä and Taponen, 2009). In some countries, CNS have become the most prevalent pathogen that cause mastitis in dairy cows, although they mostly remain in a subclinical state (Pitkälä et al., 2004; Tenhagen et al., 2006; Olde Riekerink et al., 2008; Pyörälä and Taponen, 2009). However, CNS can cause persistent infections, resulting in increased milk SCC and decreased milk production (Pyörälä and Taponen, 2009).

Bacteriological cure is the traditional method used to evaluate treatment efficacy. It is more objective than observation of clinical cure; however, it is not practical to evaluate this outcome on most farms. One drawback of this outcome is that when the observed sample before treatment is negative, it precludes the ability to assess bacteriological cure. The present study followed the unique definition suggested by Pinzón-Sánchez and Ruegg (2011) where matched PRE and POST milk from which pathogens were not recovered were classified as self cure. In the current study, treatment cure for cows from which pathogens were not recovered in samples at enrollment was 73% and was less than reported by Pinzón-Sánchez and Ruegg (2011) (86%). With the exception of farmers who use on-farm culture systems, farmers usually do not have microbiological diagnosis before initiating treatment and microbiologically negative cases are treated without regard to etiology. Consequently, including those cases in outcome assessments better represents the real scenario that farmers experience.

Researchers have reported a wide range of bacteriological cure (38–100%) for clinical mastitis caused by gram-negative pathogens (Guterbock et al., 1993; Bradley and Green, 2009; Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011; Schukken et al., 2011). The wide range may be explained because *E. coli* (bacteriological cure of approximately 85%) are more likely to respond favorably to treatments as compared with *Klebsiella* spp. (bacteriological cure of approximately 37%; Smith et al., 1985; Roberson et al., 2004). The usefulness of intramammary antimicrobials to treat animals experiencing mastitis caused by *E. coli* pathogens is questionable because of the high rate of spontaneous cure (Smith et al., 1985). Some studies have reported no difference in bacteriological cure rates for untreated cows compared with cows treated for mastitis caused by gram-negative pathogens, and the majority of antimicrobials labeled to treat mastitis have limited activity against these organisms (Jones and Ward, 1990; Pyörälä et al., 1994; Roberson et al., 2004). A multiherd clinical trial compared a treatment protocol based on on-farm culture (cases caused by gram-negative pathogens or no pathogen recovered were not treated) to a positive control group where all cases were treated with

cephapirin regardless of etiology (Lago et al., 2011). No significant difference in bacteriological cure between groups was found.

Similar to previous research, most cases of clinical mastitis were mild to moderate in severity (Nash et al., 2002; Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011); however, severe cases were excluded from analysis in these studies. Our percentage of severe cases was greater than previously reported (Nash et al., 2002; Lago et al., 2011; Pinzón-Sánchez and Ruegg, 2011), and outcomes of these cases were included in the analysis. In the present study, distribution of the severity score differed among microbiological diagnoses. Similar to data reported by Hogan et al. (1989), one-third of cases caused by gram-negative bacteria exhibited severe symptoms. In another study, gram-negative pathogens accounted for 35% of the total isolates and were the single most important cause of acute mastitis (Anderson et al., 1982). Few cases of mastitis caused by gram-positive pathogens exhibited severe clinical signs, similar to other studies (Todhunter et al., 1995; Taponen et al., 2006).

Classification of severity of disease is important for establishment of effective treatment protocols and help farmers to identify cows with a higher risk to develop bacteremia and the need for supportive therapy (Wenz et al., 2001, 2006). Bacteriological cure increased with severity, which was expected because the majority of severe cases were caused by gram-negative pathogens. In addition, our data demonstrated that the greatest milk production deviation was exhibited by severe cases. This could be the result of the local and systemic effects of endotoxin (Hogan and Smith, 2003).

CONCLUSIONS

On large Wisconsin dairy herds, environmental pathogens are the major cause of clinical mastitis and characteristics and outcomes of clinical cases depend on the pathogen causing clinical mastitis. Cases from which no microbes were recovered represent approximately 30% of all milk samples; further studies should determine optimal management of these cases. *Staphylococcus aureus* were isolated from few cases, demonstrating that this pathogen is not a widespread problem for large dairy herds in the region. Bacteriological cure was greatest for culture negative PRE samples and for cases caused by gram-negative bacteria as compared with other etiologies. The overall recurrence of another case of clinical mastitis within the 90-d follow-up period and removal from the herd was greater for cows that experienced cases caused by gram-negative pathogens compared with other etiologies. Of cows that had clinical mastitis cases caused by gram-negative pathogens, one-third experienced severe cases. Of cows that had mastitis caused by gram-positive pathogens, most cows experienced mild and moderate cases of mastitis. For first cases of clinical mastitis occurring in the enrolled lactation, etiology and severity were not associated with DIM at occurrence. Identification of pathogens causing clinical mastitis, as well as severity, is important to help in prevention programs.

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