



Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacteriological isolates

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Abstract — The objective of this study was to describe the frequency of occurrence of clinical mastitis in dairy herds in Ontario. The study group consisted of 65 dairy farms involved in a 2-year observational study, which included recording all clinical mastitis cases and milk sampling of quarters with clinical mastitis. Lactational incidence risks of 9.8% for abnormal milk only, 8.2% for abnormal milk with a hard or swollen udder, and 4.4% for abnormal milk plus systemic signs of illness related to mastitis were calculated for 2840 cows and heifers. Overall, 19.8% of cows experienced one or more cases of clinical mastitis during lactation. Teat injuries occurred in 2.1% of lactations. Standard bacteriology was performed on pretreatment milk samples from 834 cows with clinical mastitis. The bacteria isolated were *Staphylococcus aureus* (6.7%), *Streptococcus agalactiae* (0.7%), other *Streptococcus* spp. (14.1%), coliforms (17.2%), gram-positive bacilli (5.5%), *Corynebacterium bovis* (1.7%), and other *Staphylococcus* spp. (28.7%). There was no growth in 17.7% of samples, and 8.3% of samples were contaminated. Clinical mastitis is a common disease in dairy cows in Ontario; approximately 1 in 5 cow lactations have at least one episode of clinical mastitis. There is, however, considerable variation in the incidence of clinical mastitis among farms. The majority of 1st cases of clinical mastitis occur early in lactation, and the risk of clinical mastitis increases with increasing parity. Environmental, contagious, and minor pathogens were all associated with cases of clinical mastitis.

Résumé — **Mammites cliniques chez les bovins laitiers de l'ontario : Fréquence de la condition et isolats bactériologiques.** L'objectif de cette étude était de décrire la fréquence de la mammite clinique dans les troupeaux laitiers de l'Ontario. Le groupe d'étude était composé de 65 fermes laitières impliquées dans une étude d'observation qui comprenait le relevé de tous les cas de mammites cliniques et l'échantillonnage du lait des quartiers atteints. Au cours d'une lactation, l'incidence d'un lait simplement anormal était de 9,98 %, celle d'un lait anormal accompagné d'un pis dur ou enflé de 8,2 % et celle d'un lait anormal accompagné de signes systémiques de maladies reliées à la mammite de 4,4 %, le tout calculé à partir de 2840 vaches et génisses. Au total, 19,8 % des vaches ont eu au moins un épisode clinique de mammite au cours d'une lactation. Les blessures aux trayons sont survenues au cours de 2,1 % des lactations. Une bactériologie de routine a été effectuée préalablement à tout traitement sur des échantillons de lait de 834 vaches atteintes de mammites cliniques. Les bactéries isolées comprenaient du *Staphylococcus aureus* (6,7 %) du *Streptococcus agalactiae* (0,7 %) divers *Streptococcus* (14,1 %), des coliformes (17,2 %) des bacilles gram-positif (5,5 %), du *Corynebacterium bovis* (1,7 %) et d'autres *Staphylococcus* (28,7 %). Il n'y avait pas de croissance bactériennes chez 17,7 % des échantillons et 8,3 % de ceux-ci étaient contaminés. La mammite clinique est une maladie fréquente chez les vaches laitières de l'Ontario; environ une vache sur 5 au cours d'une lactation présente au moins un épisode de mammite chronique. Cependant, les fermes présentent entre elles une grande variation dans l'incidence de la maladie. La majorité des premiers cas de mammite clinique se produisent en début de lactation et les risques s'accroissent avec le nombre de lactations. Les agents pathogènes contagieux et opportunistes ainsi que ceux d'origine environnementale étaient tous associés à des cas de mammite clinique.

(Traduit par docteur André Blouin)

Can Vet J 1998; 39: 33-38

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Financial support was received from the Dairy Farmers of Ontario, Bayer Inc., and the Ontario Ministry of Agriculture, Food, and Rural Affairs.

Introduction

Mastitis is an economically important disease of dairy cattle worldwide. It is manifested by a wide range of clinical and subclinical conditions. Subclinical mastitis may be monitored using somatic cell counts. A number of countries have introduced penalty programs for high bulk tank somatic cell counts, with resultant decreases in average bulk tank somatic cell counts (1–3). Despite these decreases in subclinical mastitis, clinical mastitis continues to be a problem in many dairy herds (4–6).

Several previous studies have documented the frequency of occurrence of clinical mastitis (4–10). Considerable variation is found among the studies, which may be due to differences in the environmental conditions and management practices in the study herds (11), geographic differences, and differences in the criteria for selecting the study herds. One study of 32 commercial farms in 1 geographical region of Ontario revealed lactational incidence risks of 1.9% for teat injuries, 14.2% for mastitis requiring local treatment, and 2.6% for mastitis requiring systemic treatment (8). Bacteriology of clinical mastitis cases was not performed. No studies of the frequency of occurrence of clinical mastitis and of the bacterial pathogens involved have been conducted recently in commercial dairy herds in Ontario. This information is necessary to determine the cost of clinical mastitis to the dairy industry in Ontario, and to monitor changes over time. Therefore, the objective of this study was to estimate the incidence of clinical mastitis in dairy herds in Ontario, and to provide information on the bacterial pathogens associated with clinical mastitis.

Materials and methods

The study group consisted of 65 dairy farms located in Ontario. The dairy producers were initially identified by veterinarians participating in the 2nd Dairy Health Management Certificate Program, an intensive 2-year continuing education program for dairy veterinarians at the Ontario Veterinary College (12). The producers agreed to participate in a 2-year study that involved a field trial for a gram-negative, core-antigen, mastitis vaccine (13), and an observational study of factors associated with milk protein production (14). All of the participating producers were enrolled in official milk recording with the Ontario Dairy Herd Improvement (ODHI) Corporation.

During the study period, the producers recorded all cases of clinical mastitis on a "disease event record" provided for the study. Complete details of the record keeping and data validation are available elsewhere (14). Briefly, the occurrence of clinical mastitis was recorded as any or all of the following: teat injury, abnormal milk, a hard or swollen udder, and fever or off-feed due to mastitis. The diagnoses of clinical mastitis were provided by the producer or a veterinarian. Disease event data were collected monthly, with the assistance of ODHI customer service representatives, and entered into a commercial data base (Foxpro25, Microsoft Corporation, Washington, USA). The herds were visited regularly throughout the study by 1 technician. If at

any time during the study the producer stopped recording clinical mastitis events, the last date of accurate data recording was used to identify individual animals with appropriate data recording for inclusion in the analyses.

The frequency of occurrence of mastitis was evaluated for cows and heifers that had sufficient time to complete a 305-day lactation during the study period. Therefore, it essentially included all animals that calved during the 1st year of the study. Cows that would have had time to complete a 305-day lactation but were culled prior to 305 d in milk were included in the analyses. The information on clinical signs recorded by the producers was used to categorize the clinical mastitis event as mild (abnormal milk only), moderate (abnormal milk plus udder changes), or severe (abnormal milk plus systemic signs of illness). Teat injuries were considered separately. The occurrence of mastitis was described as lactational incidence risks. The numerator was the number of cows experiencing 1 or more episodes of clinical mastitis, and the denominator was the number of 305-day cow lactations. At the herd level, the mean farm lactational incidence risk was calculated as the number of cows experiencing 1 or more episodes of mastitis divided by the number of 305-day cow lactations for that herd.

Quarter milk samples were collected aseptically from all cases of clinical mastitis prior to treatment by the producers, although only 1st cases within a lactation were used in this analysis. Disinfection and sampling methods were discussed with the producers at the start of the study. Milk samples were frozen immediately after collection. Every 4 mo during the study period, frozen milk samples were transported to the Ontario Veterinary College in Guelph, Ontario, and cultured for bacteria using standard National Mastitis Council protocols (15). The identification of a bacterium as the pathogenic cause of the mastitis event was based on the National Mastitis Council recommendations (15). Bacteriology was performed on all milk samples taken from initial cases of clinical mastitis during a lactation, regardless of whether the cow had time to complete a 305-day lactation during the study period.

Ancillary data pertaining to parity and stage of lactation were obtained from monthly ODHI test-day records.

Results

Data collection began in May 1993 and continued until March 1995. There were 2840 cows and heifers included in the analysis of lactational incidence risks. Originally, there were 75 Ontario dairy herds enrolled in the field trial and observational study components of the project, but 10 herds had poor health records and were not included in the analysis of the incidence of clinical mastitis.

In the winter of 1994, the average herd size for the study group was 52 (range of 24 to 216). Mean herd Breed Class Average (BCA) values were 178, 179, and 179 for milk, protein, and fat, respectively. The mean herd somatic cell count, derived from individual cow values from 3 mo in the winter of 1994, was 181.7×1000 ($S_x = 10.7 \times 1000$).

Table 1. Lactational incidence risks of clinical mastitis of different severities at the individual cow level, and the range in farm means for 2840 cows and heifers on 65 dairy farms in Ontario

Severity of mastitis	Individual cow level (%)	Range in farm means (%)
Teat injury, with or without mastitis	2.1	0–18.8
Not associated with a teat injury:		
Abnormal milk only	9.8	0–37.5
Abnormal milk with a hard, swollen udder	8.2	0–28.6
Abnormal milk with systemic signs of mastitis (fever / off feed)	4.4	0–33.3
Overall (all severities, not associated with a teat injury)	19.8	0–58.3

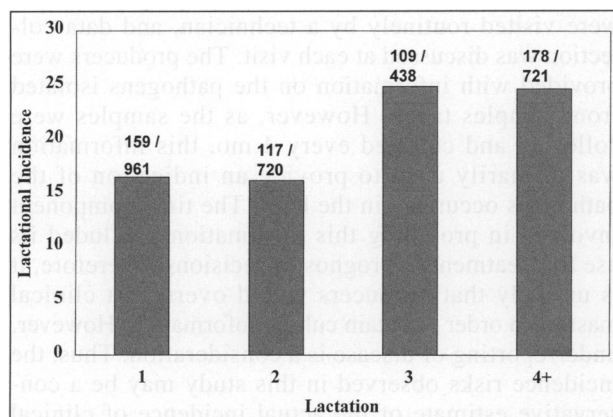


Figure 1. Lactation risk of mastitis by parity for 2840 cows in 65 dairy herds in Ontario, 1993–1995.

Table 1 shows the lactational incidence risks for clinical mastitis, by severity, at the individual cow level, and the range of lactational incidence risks of clinical mastitis at the farm level. There was considerable variation among farms in the lactational incidence of clinical mastitis. At the cow level, approximately 20% of cows experienced 1 or more clinical mastitis events in a lactation. It was possible for a cow to experience clinical mastitis of different severities during a lactation, and thus be included as a clinical mastitis case in more than 1 category. Therefore, the overall lactational incidence risk for mastitis was less than the sum of the different severity categories. The most common classification of clinical mastitis was abnormal milk only.

Figure 1 shows the lactational incidence risk of mastitis (any severity) by lactation, and the numerator and denominator used to calculate the risks. Older cows were more likely to have one or more cases of mastitis during a lactation. However, the number of cows in lactations 1 and 2 was considerably larger than the number of cows in later lactations. Therefore, the younger cows accounted for a substantial number of clinical mastitis cases.

Figure 2 shows a frequency distribution of the lactational incidence risk of 1st cases of mild and severe mastitis by days in milk. Mastitis tended to occur for the 1st time early in lactation, although 1st cases of mastitis did occur throughout lactation. There was little difference between mild and severe mastitis in the time during lactation when the 1st mastitis case occurred.

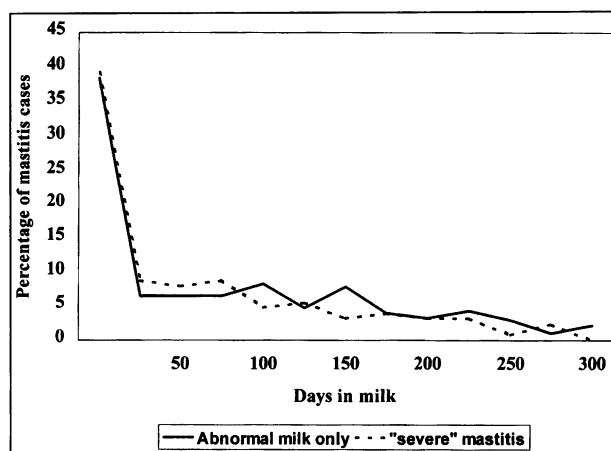


Figure 2. Frequency distribution of the incidence risk of 1st cases of mild and severe mastitis by days in milk for cows in 65 dairy herds in Ontario from May 1993 to March 1995.

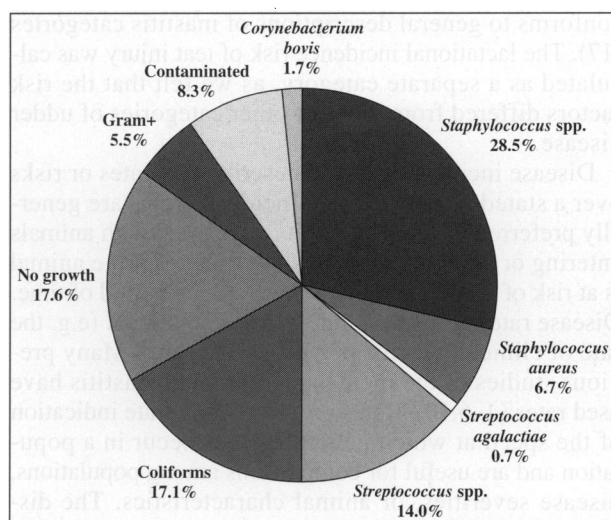


Figure 3. Frequency of bacteriological isolates from 834 clinical mastitis cases in 65 dairy herds in Ontario taken from May 1993 to March 1995.

Milk samples were available for bacteriological culture for 834 initial cases of clinical mastitis within a lactation. Figure 3 shows the percentage of clinical mastitis cases (all severities combined) associated with each of the common bacterial mastitis pathogens. Contagious mastitis pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*) were identified in 7.4% of mastitis cases. Of the 834 clinical mastitis cases cultured, 6 were positive for *Str. agalactiae*. Environmental pathogens (non-*Str. agalactiae* spp., coliforms) were identified in 31.3% of samples, and minor pathogens (*Corynebacterium bovis*, *Staphylococcus* spp. other than *St. aureus*) were identified in 30.4% of samples.

The coliform bacteria were further classified by pathogen. The specific coliform bacteria identified were *Escherichia coli* (71.4%), *Klebsiella* spp. (21.8%), *Serratia* spp. (5.3%), and *Enterobacter* spp. (1.5%).

Discussion

The herds used in the study were not randomly selected, and their production levels were slightly higher than the

provincial average (16). Therefore, the incidence of mastitis on these farms may not accurately reflect mastitis in herds in general in Ontario. However, considerable record keeping by the producers was required, and this necessitated the use of volunteer producers to form the study group. The study herds were concurrently participating in a field trial for a gram-negative, core-antigen, mastitis vaccine. The vaccine did not significantly reduce the overall incidence of clinical mastitis or the pathogen-specific incidence of clinical mastitis in this population (13). Therefore, the use of the vaccine was unlikely to have influenced the lactational incidence risks reported in this study.

Since clinical mastitis events were based on producer or veterinarian diagnosis, it is possible that the definition of clinical mastitis may have differed among farms. Clinical mastitis may manifest as a wide variety of clinical signs. However, the use of clinical signs to define the severity of clinical mastitis in the present study conforms to general descriptions of mastitis categories (17). The lactational incidence risk of teat injury was calculated as a separate category, as we felt that the risk factors differed from those of other categories of udder disease.

Disease incidence may be described by rates or risks over a stated period of time. Incidence rates are generally preferred if the population is dynamic, with animals entering or leaving the study over time, or if the animal is at risk of getting the disease over a long period of time. Disease rates always include a time component (e.g. the rate of clinical mastitis per 100 cow years). Many previous studies of the incidence of clinical mastitis have used rates (4–6,10). Rates provide an accurate indication of the speed at which new infections occur in a population and are useful for comparisons among populations, disease severities, or animal characteristics. The disadvantage of defining disease incidence using rates is that they have no direct interpretation at the animal level. By contrast, disease risk is expressed as a percentage, which has a meaningful interpretation at the animal level. Risks are used to describe disease incidence for fixed populations when the time at risk is relatively short. The short time period at risk is important due to the occurrence of competing risks. For instance, if an animal is at risk for a disease throughout lactation, and that animal dies in midlactation, it is impossible to determine whether the animal would have developed the disease of interest later in the lactation. Thus, risk is often used for diseases that occur around the time of calving, such as milk fever and retained placenta. When used to define diseases that can occur more than once during a lactation, such as mastitis, lactational incidence risk is defined as the percentage of cow lactations with at least 1 occurrence of the disease (18).

In the present study, cows entered the study and entered new lactations over time. Thus, the population was dynamic. However, by considering the date of each calving as the 1st day at risk for mastitis, and only including in the analysis those animals that had time to complete a full 305-day lactation during the study period, it was possible to fix the population. The majority of cases of mastitis occurred early in lactation (Figure 2). Thus, it may be argued that the biological

period at risk was short. Therefore, as lactational incidence risk has a meaningful interpretation for veterinarians and producers at the cow and herd level, lactational incidence risk was used to describe the occurrence of mastitis in the present study.

The pronounced differences in the incidence of clinical mastitis among herds is consistent with the findings of other reports in the literature (4–6,10). There are a number of possible reasons for these differences. There may be actual differences among producers in their ability to prevent or control clinical mastitis, in their motivation for and reliability of record keeping, and in their diagnostic ability. While it is difficult to control motivation for record keeping, the owners of herds enrolled in this study were volunteers and had agreed to participate in the necessary record keeping. The herds were visited routinely by a technician, and data collection was discussed at each visit. The producers were provided with information on the pathogens isolated from samples taken. However, as the samples were collected and cultured every 4 mo, this information was primarily used to provide an indication of the pathogens occurring in the herd. The time component involved in providing this information precluded its use for treatment or prognostic decisions. Therefore, it is unlikely that producers would overreport clinical mastitis in order to obtain culture information. However, underreporting of disease is a consideration. Thus, the incidence risks observed in this study may be a conservative estimate of the actual incidence of clinical mastitis. Differences among producers in diagnostic ability could also lead to apparent differences among farms in the incidence of mastitis. While diagnostic acuity was not examined in the present study, it has been determined that differences in diagnostic ability were not associated with incidence rates of clinical mastitis (19). Therefore, it was felt that the differences in the incidence of mastitis observed among farms represented a real difference in the ability of producers to control clinical mastitis. Some farms did not appear to have a clinical mastitis problem, while other farms had a very high incidence.

Numerous other studies have reported an increased risk of mastitis early in lactation (5–7,9,10). Dohoo *et al* (8) reported differences in the time of greatest risk based on the severity of the mastitis event; for mastitis requiring systemic therapy, the greatest period of risk was the 1st wk of lactation, whereas cows were at the greatest risk of acquiring mastitis requiring local therapy during the 1st mo of lactation. In the present study, the greatest risk of 1st acquiring mastitis occurred early in lactation, regardless of the severity of the mastitis.

The increase in the risk of clinical mastitis with increasing parity is generally consistent with previous reports in the literature (9,20,21), although one study reported the highest rates of clinical mastitis in 1st-calf heifers (5). In Ontario, the annual culling rate for ODHI herds is 31%, and cows are culled after completing an average of 2.8 lactations (22). Therefore, a substantial percentage of dairy cattle in Ontario are in their 1st or 2nd lactation. Although the risk of mastitis was lowest for 1st-calf heifers, approximately 1/3 of the animals in the study were in their 1st lactation. This group,

therefore, accounted for a large number of the cases of clinical mastitis. Thus, it is important to include heifers in mastitis control programs.

In this study, the herds were sampled without regard to the herd somatic cell count. Several previous studies investigating the bacterial pathogens associated with clinical mastitis have sampled herds based on somatic cell count (4–6). Significant differences between high and low somatic cell count herds in the percentage of clinical mastitis samples with *Str. agalactiae*, *St. aureus*, coliforms, and contamination have been reported (4). Thus, it is important to consider herd sampling methods when comparing bacteriological findings among studies.

Milk samples in this study were collected every 4 mo and, therefore, samples were frozen for various durations prior to culture. Freezing of milk samples has been shown to have an effect of the ability to isolate specific bacteria. In a study involving clinically normal and mastitic cows, cultures from milk samples frozen at -20°C for 23 d had an increased frequency of isolation of *Str. agalactiae* and *St. aureus* compared with cultures from the same samples prior to freezing (23). Using milk samples from quarters with clinical mastitis, it has been shown that freezing and an increased length of storage result in a decrease in the number of samples cultured with *E. coli*, an increase in the number of samples with coagulase-negative staphylococci, and no effect on the number of samples with streptococci or *St. aureus* (24).

Culture results from milk samples of all 1st cases of mastitis during the study period were included in the analysis. By contrast, lactational incidence risks were only calculated for cows with time to complete a 305-day lactation during the study period. Thus, it is possible that the culture results reported are biased towards overrepresentation of samples taken early in lactation. However, the majority of 1st cases of mastitis did occur early in the lactation period.

The percentage (6.7%) of milk samples with *St. aureus* found in this study was higher than the percentages reported for herds with low somatic cell counts in the northeastern United States (4,5) and lower than the percentages reported for herds with high somatic cell counts (4). The percentage of *St. aureus* samples was lower than the percentages cited for herds with low somatic counts (9.6%) (6) and a random sample of herds (14.4%) in the Netherlands (10). This may reflect geographic differences. The percentage of gram-negative *Staphylococcus* spp. in the present study was considerably higher than percentages cited in the literature for herds with low somatic cell counts (4–6,10). A study of individual cow composite milk samples from cows in 71 herds in Ontario reported that 55% of herds had at least one cow infected with *Str. agalactiae*, and the prevalence of cows infected within herd averaged 7.0% (25). The present study measured lactational incidence risk of clinical mastitis caused by *Str. agalactiae*, rather than herd prevalence. However, the results show that *Str. agalactiae* is present in some dairy herds in Ontario. The present study illustrates that environmental, contagious, and minor pathogens are all commonly associated with clinical mastitis in dairy herds in Ontario.

The percentage of milk samples that yielded no bacterial growth was somewhat less than that of other esti-

mates (4–6,10). However, no bacteria were isolated from a substantial percentage of the mastitis samples. Standard bacterial culture techniques may not be adequate to isolate all of the bacteria potentially associated with mastitis. Bacteria-negative samples may occur due to spontaneous bacterial cure, the presence of too few viable bacteria for culture techniques, inhibition of bacteria by antibiotics, or death of the bacteria after removal of the milk sample from the gland but prior to culture (26). Antigens against mastitis-causing bacteria were detected in 68% of 84 quarter milk samples from mastitis cows where no bacteria were isolated using standard culture techniques (26). The sensitivity of culturing a single quarter milk sample for *St. aureus* has been determined to be 75% (27). Collecting more than 1 milk sample from cows with clinical mastitis prior to treatment (28) or augmenting culture techniques by pre-culture incubation of samples and increased plate inoculation volumes (29) may decrease the percentage of bacteriologically negative samples.

Clinical mastitis is a common disease in dairy cows in Ontario; approximately 1 in 5 cow lactations have at least 1 episode of clinical mastitis. There is, however, considerable variation in the incidence of clinical mastitis among farms. The majority of 1st cases of clinical mastitis occur early in lactation, and the risk of clinical mastitis increases with increasing parity. Environmental, contagious, and minor pathogens were all associated with cases of clinical mastitis.

Acknowledgments

We thank the management and staff of the Ontario Dairy Herd Improvement Corporation for their assistance in the collection and data entry of the health information, and the participating producers and veterinarians for their time, enthusiasm, and commitment to this project. cvj

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