Pathogen Identification And Incidence Rates Of Clinical Mastitis On Organic And Conventional Dairy Farms

by

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ABSTRACT

**PATHOGEN IDENTIFICATION AND INCIDENCE RATES OF CLINICAL
MASTITIS ON ORGANIC AND CONVENTIONAL DAIRY FARMS**

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Thesis objectives were: 1) evaluate incidence rate of producer reported clinical mastitis

(IRCM) and predominant pathogen types on conventional and organic dairies; 2)

investigate associations between farm type, housing and access to pasture and IRCM; 3)

explore association between bulk tank somatic cell count (BTSCC) and IRCM. An

investigation on 59 dairy farms in Southern Ontario, Canada, involving organic and

conventional management systems was conducted from April 2011 to March 2012.

Coagulase-negative staphylococci were the most frequent pathogens (20.4%);

Staphylococcus aureus was the most common contagious pathogen (9.6%). The overall

mean IRCM was 24.7 cases/100 cow-years. Organic farms tended to have lower IRCM

than conventional farms (17.1 cases/100 cow-years vs. 28.1 cases/100 cow-years). In

summary, mean IRCM tended to be lower in the organic compared to conventionally

managed herds. Housing, pasture use, and BTSCC category were not associated with

overall IRCM; however, these factors did impact pathogen-specific IRCM.

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CHAPTER 1: INTRODUCTION

1.1 Definition of Mastitis

Mastitis is inflammation of the parenchyma of the mammary gland, which can result from cow exposure to a variety of infectious agents (Radostits et al., 2000). Mastitis is characterized by an array of physical and chemical changes in milk, pathologic changes to the glandular mammary tissue, and at times systemic changes within the affected animal (Radostits et al., 2000).

Mammary infections are divided into two categories, sub-clinical and clinical. Cases of sub-clinical mastitis are described as the presence of an infection without visually evident sign of local inflammation or systemic involvement (Erskine, 2011). Detection of sub-clinical mastitis can be accomplished by determining the somatic cell count (SCC) in milk of individual cows using a California Mastitis Test (CMT) or automated cell count methods (Erskine, 2011). Somatic cells include lymphocytes, macrophages, polymorphonuclear cells and some epithelial cells, all of which reflect the inflammatory response in the udder to an intramammary infection (IMI) (Schukken et al., 2003). Somatic cell counts to monitor for sub-clinical mastitis can be conducted at the quarter, cow, and herd levels (Schukken et al., 2003).

In clinical mastitis (CM) there are visible changes to the normal appearance of milk, which could include a colour change, a consistency change, or the presence of flakes, clots, and/or blood (Radostits et al., 2000). Physical changes to the udder may be present in CM cases ranging from warmth, diffuse swelling, and pain to gangrene in severe cases (Radostits et al., 2000). Chronic mastitis can result in local fibrosis and

atrophy of mammary tissue (Radostits et al., 2000). When only local signs are evident a case of mastitis is considered mild or moderate (Erskine, 2011). A case of mastitis is considered severe when systemic signs of an inflammatory response are apparent including fever, anorexia, and shock (Erskine, 2011).

1.2 Importance of Mastitis Research

Mastitis, an endemic disease (Halasa et al., 2007), is a major concern of the dairy industry for a number of reasons: (1) mastitis has deleterious effects on milk composition, yield, and quality of dairy products (Auldist and Hibble, 1998); (2) mastitis is considered a welfare concern due to the pain cows experience, especially during an episode of acute, severe mastitis (Kemp et al., 2008; Siivonen et al., 2011; Leslie and Petersson-Wolfe, 2012); and (3) mastitis is the most common production limiting disease on dairy farms worldwide and the most costly (Reyher et al. 2011). Factors which contribute to the economic impacts of mastitis, include milk production losses, diagnostic costs, treatment costs, discarded milk, increased risk of other diseases and culling of dairy animals (Halasa et al., 2007). On Canadian dairy farms in 2011, 8.7% of cows were culled as a result of mastitis, second to reproductive problem and before feet and leg issues (Agriculture and Agri-food Canada, 2012).

1.3 Mastitis Pathogens

Many different infectious agents can cause bovine mastitis. Pathogens are typically divided into two categories, contagious and environmental (Radostits et al., 2000), which are used to describe the epidemiology of the primary pathogens causing

IMI (Smith and Hogan, 1993). Contagious pathogens are ones, which spread from infected quarters to other quarters and other animals (Radostits et al., 2000). Bacteria in this category include *Staphylococcus aureus, Streptococcus agalactiae, Streptcococcus dysgalatiae* and *Mycoplasma* bovis (Diver and Peek, 2008). Contagious pathogen spread occurs mainly during the milking process through contaminated milking equipment, milker's hands, or materials used to wash or dry the udders of multiple cows (Harmon, 1996). The primary reservoir for contagious pathogens is infected cattle (Keefe, 2012). Smith and Hogan (1993) commented that contagious mastitis is primarily associated with cases of sub-clinical mastitis.

Environmental mastitis pathogens are those found in the environment that dairy cattle inhabit (Smith and Hogan, 1993). Sources of bacteria include bedding materials, soil, manure, and other organic matter (Hogan and Smith, 2001), and as a result of the wide variety of sources, cattle have the potential for constant exposure during both the dry and lactation periods (Smith and Hogan, 1993). Pathogens within the environment include coagulase-negative staphylococci (CNS)*, Trueperella pyogenes* (previously named *Arcanobacterium pyogenes*), environmental streptococci, coliforms, other gram-negative bacteria, yeast, fungi, and *Prototheca* spp. (Divers and Peek, 2008). In North America the most common environmental mastitis pathogens are coliforms and environmental streptococci (Hogan and Smith, 2012). Coliforms are a group of gram-negative bacteria that includes *Escherichia, Klebsiella,* and *Enterobacter* (Hogan and Smith, 2012). *Serratia, Pseudomonas,* and *Proteus* are considered the other gram-negative bacteria frequently found in IMI (Hogan and Smith, 2012**).**

Environmental streptococci is also a title given to a group of mastitis pathogens; bacteria within this group are *Streptococcus uberis, Streptococcus dysgalactiae,* and *Enterococcus* spp. (Hogan and Smith, 2003). *Streptococcus dysgalactiae* is a unique mastitis pathogen as it can reside in udders and the environment, and therefore it possess the characteristics of both a contagious and environmental pathogen (Smith and Hogan, 1995). Environmental pathogens are significant contributors to CM cases with often a reduced impact on bulk milk somatic cell count (BTSCC) as CM milk is commonly discarded and not added to a bulk milk tank due to its abnormal appearance and accompanying antibiotic therapy (Smith and Hogan, 2001).

Mastitis causing pathogens can be further classified into two groups: (1) major and (2) minor. Major pathogens are those commonly associated with CM (Radostits et al., 2000), often persisting in the udder for prolonged periods of time, causing damage to the udder and substantial inflammation, which can be detected by an increase in SCC (Reyher et al. 2012). Minor pathogens are ones that do not usually cause CM (Radostits et al., 2000). *Streptococcus agalactiae* and *Staphylococcus aureus* are considered major contagious mastitis pathogens due to their large deleterious effects on milk quality, production, and cow SCC (Keefe, 2012). Major environment pathogens are coliforms, of which the most common species are *Escherichia coli* and *Klebsiella* spp. (Schukken et al., 2012). Traditionally CNS has been considered a minor pathogen, however, Taponen et al. (2006) suggested that CNS is becoming a predominant pathogen causing mastitis. In a study by Schukken et al. (2009), ~9% of cows were infected with CNS. Researchers concluded that IMI with CNS result in a moderate increase of SCC, but relatively small when compared to cows infected with major pathogens (Schukken et al., 2009). CNS was

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found to not likely cause an important increase in BTSCC for herds with poor milk quality, but may be an important contributor to SCC in herds with already low BTSCC (Schukken et al., 2009).

It has been suggested that observing SCC could give an indication to the type of pathogen a cow is infected with (NMC, 1997). Although cows with a SCC >250,000 cells/ml could be suspected of harboring an infection due to a major mastitis pathogen while cows with an SCC <250,000 cells/ml likely are not (NMC, 1997), researchers have found that SCC levels and durations are variable based on pathogen type (de Haas et al., 2004; Green et al., 2004). Therefore, only reviewing cow SCC to determine type of mastitis pathogen involved in a cases of mastitis may be misleading. Using a cut off of SCC 200,000-250,000 cells/mL to distinguish between infected and uninfected quarters (Schukken et al., 2003) may be a more appropriate use of SCC in on-farm monitoring of udder health.

1.4 Risk Factors for Clinical Mastitis During Lactation

Many studies have been conducted looking at the risk factors for CM on dairy farms (Barkema et al., 1999; Green et al., 2007; Breen et al. 2009). Commonly, CM risks are categorized into three levels: (1) herd, (2) cow, and (3) quarter. Which factors hold the greatest importance appears to vary between studies; this variability may result for multiple reasons including study location, predominant pathogen types identified, data collection methods (e.g. historical record analysis versus current on-farm collection), and enrolled herds.

1.4.1 Herd

Certain herd factors can increase the risk of CM due to specific mastitis pathogens. Results from Barkema et al. (1999) indicated that it may be difficult to find herd-level risk factors for *Streptococcus uberis;* however, it was clear that management factors strongly affected CM caused by *Escherichia coli, Streptococcus dysgalactiae,* and *Staphylococcus aureus.*

For CM risk factors at the herd level, housing type has been shown to impact incidence (Olde Riekerink et al., 2008; Schukken et al., 1990). Tie stall housing was associated with higher incidence rates of CM when compared to free-stall housing systems (Olde Riekerink et al., 2008); this may result from particular management strategies used in each housing system. Pathogen specific incidence rate of clinical mastitis (IRCM) can be related to housing type. Klebsiella was more frequently isolated from CM samples of cows kept in free-stall systems than tie-stall housing (Olde Riekerink et al., 2008). *Staphylococcus aureus, Streptococcus uberis, CNS* and other Streptococcal IRCM were higher in tie-stall herds than free-stall herds (Olde Riekerink et al., 2008).

Schukken et al. (1990) found poor stall hygiene correlated with both the rate of CM and the cleanliness scores of cow for herds with low BTSCC. Poor stall hygiene facilitates cow exposure to microorganisms as teats are in close contact with stall surfaces or bedding (Elbers et al., 1998) thereby increasing the chances for pathogens to infiltrate the udder and cause CM; this is particularly true of environmental pathogens such as

Escherichia coli (Schukken et al., 1991; Elbers et al., 1998) and is also important with contagious pathogens such as *Staphylococcus aureus* (Elbers et al., 1998).

Aspects of milking management have been found to influence risk of CM. Stripping foremilk before attaching the milking unit was found to increase the risk for CM (Peeler et al., 2000). Foremilk stripping has been specifically associated with higher rates of CM cause by *Escherichia coli* (Elbers et al., 1998) and *Staphylococcus aureus* (Schukken et al., 1991; Elbers et al., 1998). With forestripping, pathogens may be released into the environment increasing exposure of uninfected cows to mastitis causing bacteria or may be passed between quarters and cows by contaminated hands of those milking (Peeler et al., 2000). It is reasonable to consider that herds which forestrip may not have a truly increased incidence of CM, rather as Peeler et al. (2000) hypothesized checking foremilk could result in detection of mild cases of mastitis that would otherwise go unnoticed therefore increasing the overall number of CM cases observed.

The use of teat disinfection as part of a milking routine has also been associated with increased levels of CM in herds with low BTSCC (Schukken et al., 1990; Elbers et al., 1998; Barkema et al, 1999). Barkeam et al. (1999) found no association of postmilking teat disinfection with IRCM in herds with high BTSCC. Schukken et al. (1990) suggested that the association of teat disinfection in herds with increased rates of CM may be cause and effect; herds have increased IRCM already, therefore they employ teat disinfection to try and combat the CM problem among their cows. Research has also suggested that greater use of teat disinfection after milking may lead to a reduction in quarters infected with minor pathogens, thereby increasing the risk of infection by major pathogens (Schukken et al., 1989). Major pathogens are more likely to cause CM, which

is then detected by producers increasing their overall farm IRCM. Barkema et al. (1999) reported that the overall increased IRCM in herds using postmilking teat disinfection was associated with *Escherichia coli.* Although some studies have shown postmilking teat disinfection to be associated with IRCM, teat disinfection is still considered an important defense against IMI, typically for contagious mastitis pathogens (Keefe, 2012).

Milking machine factors which influence teat health (e.g. increase teat end hyperkeratosis) have been associated with IRCM caused by *Escherichia coli, Streptococcus dysgalactiae* and *Streptococcus uberis* (Barkema et al., 1999). Machine factors included high vacuum pressure, wet premilking treatment which caused movement of teat liners and short squeeze phase of pulsation (Barkema et al., 1999).

1.4.2 Cow

There are several aspects of individual cows that may predispose them to cases of CM. Increasing parity has been shown to elevate the risk of CM (Green et al., 2007; Breen et al., 2009). Breen et al. (2009) suggested that increased risk of mastitis in older animals might relate to concurrent health issues, which are often present (e.g. lameness). Green et al. (2007) commented that anatomical changes to the teat end or changes in the immune status of a cow may result with aging, thereby increasing susceptibility to infection by mastitis pathogens. Chronic mastitis infections may be present in older animals resulting in an accumulated risk of clinical disease (Green et al., 2007). As well, a positive association has been demonstrated between high yielding dairy animals and IRCM (Bigras-Poulin et al., 1990; Peeler et al., 2000; Barnouin et al., 2005); although these studies did not investigate the role parity played in yield and IRCM, it would be

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reasonable to consider that parity may play as role as multiparous cows often have higher production than primiparous animals.

Cow cleanliness has been related to IRCM (Ward et al., 2002; Breen et al., 2009). Breen et al. (2009) found that very dirty udders were significantly associated with an increased risk of CM. Greater amounts of debris on the udder or hind legs (which the udder may contact when the cow lays down), increases exposure of the cow to possible mastitis pathogens.

Somatic cell count is considered to be an important risk factor for CM (Steeneveld et al., 2008). Cows with elevated composite SCC (>200,000 cells/mL) had 2-4 fold-increased risk of developing CM during the lactation than cows with low composite SCC (van den Borne et al., 2011).

Additionally, decreasing month of lactation was associated with an increased risk of CM (Breen et al., 2009). Cows with a SCC >199,000 cells/mL at their first test day were more likely to develop CM within the first 30 days of their lactation (Green et al., 2007; Breen et al., 2009). Breen et al. (2009) suggested that cows who calved with an increased SCC will be at greater risk of CM because they may be in the early stages of an active infection or a subclinical mastitis episode may be present, which has the potential to become clinical.

1.4.3 Quarter

When examining risk at the quarter level, severe hyperkeratosis of the teat end has been associated with an increased risk of CM (Breen et al., 2009). Teat orifice and teat canal health are greatly influenced by milking equipment factors (Barkema et al.,

1999). It is logical that when teat health is compromised the IRCM increases as teat orifice and teat canal are the first line of defense against the entrance of mastitis causing pathogens into the mammary gland (Barkema et al., 1999). The risk of bacterial colonization of the streak canal and subsequent development of CM is increased with severe disruption to the normal anatomy of the teat orifice (Breen et al., 2009).

High peak milk flow rates (>2L/min) and decreased quarter yield (<1L/quarter/milking) have been associated with increased risk of CM (Hammer et al., 2012). Hammer et al. (2012) suggested the high peak flow rates might be related to teat canal muscle weakness, which results in decreased defense against invasion of mastitis pathogens. Reduced quarter yield preceding CM cases is likely a result of increased udder pathology (Hammer et al., 2012).

Peeler et al., (2000) found that herd average lactational yield >7500 L was significantly associated with leaking milk outside the parlor. Leaking milk on entering the parlor or at other times were important risks for CM (Peeler et al., 2000). Leaking milk may increase levels of mastitis pathogens distributed in the environment causing greater risk of exposure to other cows (Peeler et al., 2000). As well, cows that leak milk may have increased susceptibility for pathogens to invade the udder due to wide teat canals (Lacy-Hulbert and Hillerton, 1995).

Somatic cell count has not only been associated with IRCM at the cow level but at the quarter level as well. Green et al. (2004) concluded that quarters with low SCC (<41,000 cells/mL) were at greater risk of CM in the next month than quarters with a SCC in the range 41,000-100,000 cells/mL. Quarters with a SCC >200,000 cells/mL were

also found to be at greater risk of CM in the next month (Green et al., 2004). The results suggest that quarter immune factors influence risk of CM (Green et al., 2004).

1.5 Control of Clinical Mastitis

Udder health programs are important for a wide variety of reasons, including animal welfare issues due to the pain CM can incite and milk quality for dairy products destined for human consumption, which is also related to SCC limits and the risk of antibiotic residues (Schukken et al., 2003). Mastitis control programs are based on prevention of new infections and elimination of existing infections (Ruegg, 2003). Rodrigues et al. (2005) reported that mastitis control programs focus on adoption of research-based practices and management principles that reduce the level of exposure to mastitis pathogens. Management principles could include: milk culturing to identify causative agents thereby facilitating appropriate management strategies such as suitable antibiotic treatment or isolation of cows infected with a contagious pathogen (Cressier and Bissonnette, 2011); maintaining good milking practices (e.g. forestripping, pre- and post-dip use, always wearing rubber gloves, regular milker training) and milk equipment maintenance (e.g. maintenance as recommended by the manufacturer) to reduce teat-end exposure to bacteria and reduce teat damage (Rodrigues et al., 2005); culling cows with 3 or more occurrences of CM within a lactation which may decrease the IRCM in herds by eliminating potential spread of contagious pathogens and removing cows that appear to have increased susceptibility to developing cases of CM (Barnouin et al., 2005).

The National Mastitis Council (2001) developed a 10-point recommended mastitis control program: 1) establishment of goals for udder health; 2) maintenance of a clean,

dry, comfortable environment; 3) proper milking procedures; 4) proper maintenance and use of milking equipment; 5) good record keeping; 6) appropriate management of CM during lactation; 7) effective dry cow management; 8) maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows; 9) regular monitoring of udder health status; 10) periodic review of mastitis control program. The 10-point program puts focus on areas of risk for developing mastitis. Farms which implement on-farm mastitis control plans have been shown to significantly reduce the proportion of cows affected with CM when compared to control farms with no mastitis control plan (Green et al., 2007).

1.6 Clinical Mastitis in Canada

Scientific literature is readily available on the status of CM in dairy production systems throughout the world. When reviewing studies researchers must keep in mind that herd management practices often differ among countries as a result of varying environmental conditions (Olde Riekerink et al., 2006), regulatory requirements [e.g. in Sweden only veterinarians are permitted to initiate antibiotic treatments for disease (Nyman et al., 2007)], geographic variation of mastitis pathogens (Olde Riekerink et al., 2008), and case selection criteria and possibly attitudes of producers participating in field studies. To specifically address the status of CM in Canada, several studies have investigated incidence rates and reported the following means: 26.3 cases per 100 cow-years at risk (Thompson-Crispi et al., 2013); 0.22 cases per cow-year (Reyher et al., 2011); 23.0 cases per 100 cow-years (Olde Riekerink et al., 2008); 21.8 cases per 100 lactations (McLaren et al., 2006); 5.6 and 10.5% lactational incidence rate for first

lactation and fifth or greater lactations respectively (van Dorp et al., 1999); 19.8 lactational incidence risk (Sargent et al., 1998); and 0.37 cases per animal-year (Meek et al., 1986). The studies by Olde Riekerink et al. (2008) and Reyher at al. (2011) were conducted at the national level and the remaining studies were limited to more regional locations within the province of Ontario (Meek et al., 1986; Sargent et al., 1998; McLaren et al., 2006) and British Columbia (van Dorp et al., 1999). Sargeant et al. (1998) and Olde Reikerink et al. (2008) were specifically interested in incidence of CM in contrast to the work by McLaren et al. (2006), van Drop et al. (1999) and Meek et al. (1986) that had broader objectives of identifying multiple diseases in dairy herds, one of which being CM.

Overall, it is difficult to directly compare studies because of differences in study design (Olde Riekerink et al., 2008). Sargeant et al. (1998) determined IRCM using only full 305-day lactations; it was suggested that the criteria used by Sargeant et al. (1998) could have underestimated true IRCM as cows with CM are more likely to be culled before completing a lactation (Olde Riekerink et al., 2008). The slightly lower IRCM reported by McLaren et al. (2006) could be related to inclusion of data from farms with as few as 3 mo of disease reporting; therefore a clear representation of yearly disease incidence may have been under evaluated. Van Drop et al. (1999) reported results well below that of the other Canadian studies, which could be a result of the study including only the first occurrence of CM within each of the three defined time periods (0-30, 31-150 and 151-365 d postpartum), thus excluding recurrent cases from the incidence rate determined. The mean IRCM reported by Olde Riekerink et al. (2008) ranged from 7.6 cases per 100 cow-years to 31.6 cases per 100 cow-years in Manitoba and Ontario,

respectively. The advantage of the national survey by Olde Riekerink et al. (2008) is that it provides an extensive view of the IRCM across Canada and suggests why differences may exist between provinces. For example the high IRCM in Ontario herds could be explained by the large use of tie-stall barns and the associated management with that type of housing system as compared to free-stall housing which was more frequently used in the Western provinces (Olde Riekerink et al., 2008).

Observational studies such as these that rely on producer reporting have the potential of misrepresented disease incidence as producer may under-report for a variety of reasons (Bartlett et al., 2001) or be more inclined to record or remember episodes of clinical treatment rather than disease (Sato et al., 2005). All of the Canadian studies discussed here had the same challenge of reporting biases, as each study used producer and/or veterinarian identified cases of clinical disease and producer records for reporting.

Pathogens causing CM in Canadian dairy herds have been reported in several studies (Sargeant et al., 1998; Olde Riekerink et al., 2008; Reyher et al., 2011). Reyher et al. (2011) found the most commonly isolated pathogens causing CM were *Staphylococcus aureus* (13%), *Escherichia coli* (11%); *Enterococcus spp.* (8.0%). In earlier work *Staphylococcus aureus* was identified in 6.7% of CM cases and *Staphyloccus* spp. accounted for 28.5% of CM pathogens (Sargeant et al., 1998). Sargeant et al. (1998) found coliforms accounted for 17.1% of bacteria isolated from CM cases; specific coliform bacteria cultured were *Escherichia coli* (71.4%), *Klebsiella* spp. (21.8%), *Serratia* spp. (5.3%) and *Enterobacter* (1.5%). Olde Reikerink et al. (2008) also reported pathogen specific IRCM. The mean IRCM of the major contagious mastitis pathogens *Staphylococcus aureus* and *Streptococcus dysgalactiae* were lowest in the Western

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provinces (1.80 and 0.27 cases per 100 cow-years respectively) and highest in Quebec (3.98 and 1.69 cases per 100 cow-years respectively) where most herds were housing in tie-stall barns; however tie-stall housing was not significantly associated with barn type (Olde Reikerink et al., 2008). Cows in tie-stalls had a higher mean IRCM for *Staphylococcus aureus* (4.04 cases per 100 cow-years), *Streptococcus uberis* (2.19 cases per 100 cow-years), CNS (1.58 cases per 100 cow-years) compared to free-stall housing systems (1.62, 0.67 and 0.68 cases per 100 cow-years, respectively) (Olde Reikerink et al., 2008). Cows housed in free stalls had higher *Klebsiella spp*. IRCM (1.00 cases per 100 cow-years) than those in tie-stall barns (0.40 cases per 100 cow-years) (Olde Reikerink et al., 2008). Overall, these studies indicate the both contagious and environment pathogens are often responsible for CM on Canadian dairy farms.

1.7 Organic Dairy Industry in Canada

The Canadian organic standards (National Standard of Canada, 2011) define organic agriculture as: "a holistic system of production designed to optimize the productivity and fitness of diverse communities within the agro-ecosystem including soil organisms, plants, livestock and people". Specifically, when taking livestock production into consideration, organic philosophies pronounce that importance should be placed on preventative measures to maintain animal health (Tikofsky, 2005), reduce animal stress (Tikofsky, 2005; Ruegg, 2009), and increase opportunities for animals to perform species-own behaviours (Tikofsky, 2005;Wagenaar et al., 2011). These principles are upheld within the Canadian organic standards (National Standard of Canada, 2011), and are mandatory for certified organic producers to follow.

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Organic agriculture is a growing industry; between 2005 and 2006 sales of certified organic foods increased in Canada by 28% (Kendrick, 2009), with an average annual growth rate of 20% over the past few years (Agriculture & Agri-food Canada, 2012). Organic dairy product sales accounted for 18% of total certified organic packaged food sales in 2008, and the number of organic dairy producers has more than doubled since 2003, resulting in 218 producers generating 937,137 hectoliters of milk (Agriculture & Agri-food Canada, 2012). Consumers are considered the major driving force for this growth. Studies indicate the main motivation behind consumer purchase of organic products include: personal health, perceived product quality, decreased chemical residues, product taste and freshness, and a perception that organic farming is less damaging to the natural environment (McEachern and McClean, 2002; Pearson et al., 2010). Animal welfare also affects consumer choice; however it is less commonly mentioned as a reason why shoppers purchase organic products (Pearson et al., 2010). Notwithstanding growth within the industry, organic milk accounts for only 1.19% of total dairy output in Canada (Agriculture & Agri-Food Canada, 2012).

1.8 Mastitis in Organic versus Conventional Dairies

While knowledge gained from mastitis risk factor research conducted on conventional farms can transfer to organic systems, it is important that research including organic systems is conducted (Vaarst and Enevoldsen, 1997; Hovi and Roderick, 2000; Pol and Ruegg, 2007), as variations exist between organic and conventional production systems.

Pasture use is not unique to organic systems. The difference between organic and conventional farm pasture use is that the Canadian organic standards (National Standard of Canada, 2011) require livestock to have access to pasture during the grazing season and open air at other times, weather permitting and conventional producers are not compelled to use pasture by regulatory standards. The amount of pasture used can differ between organic and conventional farms. Sato et al. (2005) found that 50% of organic producers used intensive rotational grazing, whereas intensive grazing was used by only 7% of conventional producers that allow cows access to pasture.

Pasture access helps to satisfy the organic philosophy that cattle should be able to express natural behaviours. Further, studies have indicated that the risk of CM decreases when cows have pasture access (Barkema et al., 1999; Green et al., 2007). Cows housed in confinement systems had 1.8 times more CM and were 8 times more likely to be culled for mastitis than cows in a pasture based system during a 4 years study in North Carolina, USA (Washburn et al., 2002). In the study by Barkema et al. (1999), decreased CM risk was specifically associated with cows that had pasture access at night and was not a blanket statement for all pasture use. Lower rates of CM were found on farms where cows were at pasture during the dry period (Green et al., 2007). Reduced IRCM was related to a certain pasture rotation plan that included grazing the land for a maximum of 2 weeks and allowing 4 weeks of rest before grazing again (Green et al., 2007). It is thought that pasture conditions provide a cleaner environment for cows (Green et al., 2007); however, the study by Green et al. (2007) illustrates that if specific pasture management is not in place, pasture contamination may occur.

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Another main management factor that differs between organic and conventional dairy systems is the amount of forage fed, organic systems providing a larger quantity of forage (Hamilton et al., 2006). In Canada, organic standards require 60% of ruminants dry matter intake to come from forage (National Standard of Canada, 2011), leading to a smaller proportion of concentrates in their diet. Past research has suggested that udder health may be improved with lower levels of grain and concentrates in the diet of dairy cows (Hamilton et al., 2006). In addition, high forage may result in lower milk production, as had been observed in organic farms (Hamilton et al., 2006; Sato et al., 2005). This decrease in production may be advantageous for producers from the standpoint of mastitis risk, as high levels of milk production have been found to be positively associated with IRCM (Peeler et al., 2000).

Organic systems are not always associated with decreased rates of mastitis. Some organic management strategies employed elevate risk of CM. Ruegg (2009) reported that organic farms in the United States tend to be smaller and are more likely to utilize tie stalls, two risk factors that have been associated with increased BTSCC and risk of CM (Rodrigues et al., 2005; Olde Riekerink et al., 2008). To satisfy the organic philosophy of promoting natural behaviours, calves are often allowed to nurse their dam for an extended period of time or are placed with a nurse cow. Allowing calves to move freely within lactating cow housing may result in calves nursing from multiple cows, which could be a vehicles to spread contagious mastitis between infected and non-infected animals (Tikofsky, 2005). Green et al. (2007) found that cows had an increased rate of CM on farms where calves had access to suckle from cows other than their dam; these researchers hypothesized that access to suckle from multiple cows facilitated pathogen

spread.

Fly control methods available to organic producers are also limited by the Canadian Organic Standards (National standard of Canada, 2011), and require organic producers to utilize alternative fly control methods to conventional systems. This may be of particularly important on organic farms with mastitis due to contagious pathogens, as horn flies have been shown to transmit *Staphylococcus aureus* (Owens et al., 1998).

A major limitation in organic systems is the use of antibiotics. In Canada antibiotics are permitted if preventative measures fail to control disease (National Standard of Canada, 2011). Antibiotic withdrawal times (WDT) are double the labeled milk WDT or 30 days, whichever is longer (National Standard of Canada, 2011). A cow treated more than twice in a year will lose her organic status until she goes through a 12-month transition period to regain her organic status (National Standard of Canada, 2011). In addition to organic standards limiting producer use of antibiotics, farmer philosophy is a factor impacting antibiotic use. Vaarst et al. (2006) found that even when antibiotics are permitted, the choice to use them was influenced by the farmers desire to reduce overall antimicrobial treatments.

Restrictions on antibiotic use appear to be particularly important on mastitis risks around the dry period (Hovi and Roderick, 1998) as dry period management strategies have a key influence on the rate of CM during the next lactation (Green et al., 2007). A study in the United Kingdom found significantly more CM during the dry period in organic herds where routine use of dry cow treatment is prohibited (Hovi and Roderick, 2000). In West Germany researchers compared SCC between organic and conventional dairy herds around the dry period and found that organic cows more frequently had

elevated SCC (Muller and Sauerwein, 2010); this is important as the risk of CM increases with elevated SCC (>199,000 cells/mL) (Green et al., 2007).

Bradley and Green (2001) demonstrated the efficacy of using an IMI antibiotic dry cow treatment to prevent CM in the next lactation; they looked specifically at gram-negative CM cases. Bhutto et al. (2010) found the use of an internal teat sealant reduced the incidence of CM when compared to untreated control cows. Given limited use of dry cow treatments, organic producers must turn to alternative treatment and prevention strategies. Homeopathy is the most commonly utilized alternative (Hovi and Roderick, 2000); however it is important to note that research is limited on the effectiveness of alternative treatments (Ruegg, 2009). Where antibiotic use is limited, good udder health must focus on prevention and management strategies. These strategies include maintaining a clean, dry, comfortable environment, segregation of contagious mastitis cows, instituting biosecurity and culling guidelines, as well as ensuring proper milking procedures and implementing a plan to regularly monitor udder health (Tikofsky, 2005).

1.9 Thesis Objectives

Organic dairy production is a growing industry in Canada. Currently research has not specifically reported incidence rates or pathogen types of clinical mastitis on certified organic Canadian dairies. While there is much clinical mastitis research in organic management systems outside of Canada, Ruegg (2009) cautions against comparison of studies from different countries as the organic standards producers are required to maintain differ widely. Therefore it is important to investigate clinical mastitis on Canadian organic dairy farms.

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The objectives of this thesis were to: 1) evaluate incidence rate of producer reported clinical mastitis (IRCM) and predominant pathogen types on conventional and organic dairies in Southern Ontario, Canada, 2) investigate the associations between farm type, housing type and access to pasture for lactating animals and incidence rate of clinical mastitis, and 3) explore the association between bulk tank somatic cell count (BTSCC) and herd and pathogen-specific IRCM.

CHAPTER 2: PATHOGEN IDENTIFICATION AND INCIDENCE RATES OF CLINICAL MASTITIS ON ORGANIC AND CONVENTIONAL DAIRY FARMS

2.1 Introduction

Mastitis is recognized as the most common and costly production disease on dairy farms worldwide (Reyher et al., 2011), resulting in production losses for producers (Auldist and Hibble, 1998), decreased milk quality (Barbano et al., 2006), and negatively impacting cow welfare by inciting pain (Kemp et al., 2008; Siivonen et al., 2011; Leslie and Petersson-Wolfe, 2012), which is most obviously observed during episodes of severe clinical mastitis.

Mastitis control can be difficult regardless of system type (organic or conventional) under which the farm is managed (Marley et al., 2009). Mastitis management in organic herds does pose a particular challenge because of the restrictions on antibiotic use (Marley et al., 2009). Producers must alter treatment strategies from conventional industry norms to comply with organic regulations (Ruegg, 2009); further Ivermeyer et al. (2012) cited that low veterinary medicine inputs are important aims in organic livestock farming.

Some concerns have been expressed particularly regarding the welfare of cattle in organic systems (Vaarst et al., 2001) due to the restricted use of antibiotics and the possible delay in treatment (Marley et al., 2009; Hovi et al., 2003). Sato et al. (2005) commented that culling due to intramammary infections might be higher on organic dairies when compared to conventional due to antibiotic treatment restrictions. Studies investigating udder health on organic dairy farms are sparse (Tikofsky, 2005) and those studies, which compare organic to conventional, have conflicting results: lower IRCM

(9.1 and 14.7 cases per 100 cows in organic and conventional herds, respectively) and lower udder disease score in organic herds compared to conventional (Hamilton et al., 2006); higher SCC (increased by ~50,000 cells/mL) on organic farms compared to conventional farms (Nauta et al., 2006); no difference in any measure of udder health between organic and conventional herds (Fall et al., 2008).

To date, studies that have surveyed clinical mastitis rates and pathogens on Canadian dairies have not specifically included certified organic farms (Sargeant et al., 1998; McLaren et al., 2006; Olde Riekerink et al., 2008). The IRCM determined has been similar across Canadian studies. The rates reported are 20% lactational incidence risk (Sargeant et al., 1998), 22% lactational incidence rate (McLaren et al., 2006), 23 cases per 100 cow-years at risk (Olde Riekerink et al., 2008) and 26.3 cases per 100 cow-years at risk (Thompson-Crispi et al., 2013). The IRCM reported by Van Dorp et al. (1999) were low at 5.6 and 10.5% lactational incidence rate for first lactation and fifth or greater lactations respectively; the low level of clinical mastitis in this study could be attributed to the small group of herds enrolled (n=32).

There is value in knowing if certain management strategies contribute to herd IRCM and pathogen-specific IRCM in conventional and organic herds. Researchers have indicated that the risk of clinical mastitis decreased when cows had pasture access at night (Barkema et al., 1999) and with a specific rotation pattern of pasture use during the dry period (Green et al., 2007). Hamilton et al. (2006) determined that the main management factor that differed in organic from conventional systems was the amount of forage fed. Higher amounts of forage were fed on organic farms, which may relate to improved udder health (Hamilton et al., 2006). In addition, high forage may result in

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lower milk production, as had been observed in organic farms (Hamilton et al., 2000; Sato et al., 2005**).** This decreased production can be advantageous for producers from the standpoint of mastitis risk, as high levels of milk production have been positively associated with IRCM (Barkema et al., 1999). Housing type is another management factor that has also been associated with IRCM where herds housed in tie-stalls had higher rates than free-stall housed herds (Olde Riekerink et al., 2008).

The objectives of this study were to: 1) evaluate incidence rate of producer reported clinical mastitis (IRCM) and predominant pathogen types on conventional and organic dairies in Southern Ontario, Canada, 2) investigate the associations between farm type, housing type and access to pasture for lactating animals and incidence rate of clinical mastitis, and 3) explore the association of bulk tank somatic cell count (BTSCC) with herd and pathogen-specific IRCM.

2.2 Materials and Methods

2.2.1 Herd Selection

Mail-outs through CanWest DHI (Guelph, Ontario, Canada) were sent to 552 producers in Southern Ontario who resided within 2.5 hours of the University of Guelph (Guelph, Ontario, Canada). An initial questionnaire in the mail-out was used to identify interested producers. The response rate was 19%. From the responders, 59 herds were randomly selected based on location of herd relative to the University of Guelph (Guelph, Ontario, Canada), herd size, housing type of lactating animals and, willingness to participate that was confirmed by a telephone conversation with the producer. Herds (Table 2.1) were placed into 1 of 6 categories: certified organic herds with loose housing;

certified organic herds with tie-stall housing; conventional herds with loose housing and access to pasture for lactating animals; conventional herds with loose housing and no access to pasture; conventional herds with tie-housing and access to pasture for lactating animals; conventional herds with tie-housing and no access to pasture. Pasture was considered present when cows had regular access to a grass-based field with the opportunity to graze. While specific details of pasture usage was not available, it was known that the amount of pasture used by producers ranged between farms. Free-stall and pack barns were grouped together into the category of loose housing for purposes of this paper. All participating conventional herds consisted predominantly of Holstein cows; occasionally a small number of other breeds (e.g. Jersey, Ayrshire) were found in these herds. Organic herds had greater breed variability of milking cows. One organic farm milked strictly Brown Swiss, and another Jersey cattle. The other organic herds were composed of primarily Holstein cows, with a mixture of other breeds included (e.g. Ayrshire, Brown Swiss, Jersey, Montbéliard) and/or crossbred animals. Herds participated in the study for a 12-mo period between March 2011 and May 2012. All herds remained enrolled for the entire study duration.

2.2.2 Sampling

Participating producers were asked to aseptically collect milk samples from every quarter demonstrating signs of clinical mastitis before treatment was given. Clinical mastitis was defined to the producers as any change to the normal appearance of milk, which could include flakes, clots, blood or a watery consistency. Sample collection techniques were discussed with each producer during an initial farm visit and a written

protocol was provided along with all sampling materials. Producers were asked to record the date the sample was taken, cow identification, and which quarter (s) was sampled. In addition, the producer was asked to add a mastitis severity score (MSS) (Sargeant et al., 1998), to indicate severity of infection as follows: MSS1- quarter normal and abnormal milk; MSS 2- quarter swollen and abnormal milk; MSS 3- quarter swollen, abnormal milk and cow systemically ill (e.g. off feed, fever, down, etc.). The samples were stored in a freezer on-farm (predominantly chest-freezers were used) and were collected at least once every 8 wks. Samples were then given a sample number, compiled into batches, and shipped overnight on ice to the University of Calgary (Calgary, Alberta, Canada) for bacteriological culture.

Cow and herd specific information (e.g. cow age, calving date, lactation number, removal from herd date and reason, monthly cow SCC and BTSCC, and monthly milk production) were obtained from CanWest DHI and the Dairy Farmers of Ontario (Mississauga, Ontario, Canada). CanWest DHI information was not available for 3 herds, as they were not enrolled with the organization.

2.2.3 Laboratory Analysis

National Mastitis Council standards (Hogan et al., 1999) were used when performing the bacteriological cultures of all milk samples. An aliquot of 0.01 mL of a sample was plated on Columbia Agar with 5% sheep blood (BD Cat. # 221263) and incubated for 48 hours at 37^0C. Colonies were examined for hemolytic activity and the number of colony forming units for each sample was counted. Gram staining was performed. Gram-positive bacterial cells were further characterized by the catalase test

(Catalase Test Reagent, PML microbiologicals Cat. # R6452-30 ml). Gram-positive

cocci, which were catalase-positive, DNase positive (DNase Test Agar with Methyl

Green, BD, Cat. # 222020) and tube coagulase positive (Coagulase Plasma 25 mL,

Remel, Cat. # R21052), were identified as *Staphylococcus aureus*. Gram-positive cocci,

which were catalase negative, were identified as *Streptococcus* spp. These isolates then

underwent species differentiation tests including growth of isolates on Bile Esculin Agar

Slants (BD, Cat. # 221409) in Salt Growth Media (BD, Cat. # 297189), and in Phenol

Red Media containing Inulin and Raffinose (Hardy Diagnostics, Cat. # Y308 and Y 312).

Detection of hydrolysis of sodium hippurate was also performed (Ninhydrin reagent,

Remel, Cat. # R21238, Sodium hippurate hydrate, Sigma, Cat. # H9380-25G).

Streptococcus spp. Groups B, C, and G were identified using the Streptococcal-Select

Grouping Kit (Inter Medico, cat. # PL.041C). Gram-positive rod-shaped bacteria, which

were catalase-negative, were identified as *Trueperella pyogenes*. Gram-positive rod-

shaped bacteria, with a catalase-positive test were identified as *Bacillus* spp. or

Corynebacterium spp. depending on colony appearance.

Gram-negative rod-shaped bacteria first underwent an oxidase test (BD, BBL

DrySlide Oxidase, Cat. # 231746). Oxidase positive bacteria were identified as Other

Gram-negative rods or *Pseudomonas* spp. depending on colony appearance. Oxidase

negative bacterial isolates went through further testing including growth on MacConkey

II agar with MUG (BD, Cat. # 221938), Levine EMB agar (BD, Cat. # 221170), and

inoculation onto Simmons Citrate Agar Slant (BD, Cat. # 221026) and Urea Agar Slant

(BD, Cat. # 221096) and Motility Test Medium (BD, Cat. # 221509). Additionally, indole

spot test was performed (Indole Spot test reagent, PML microbiologicals, Cat. # R6510-

30mL). These tests were done to differentiate *Escherichia coli, Klebsiella* spp. and

Enterobacter spp.

Colony-forming units (CFU/ mL) of each bacterial species identified in a sample were used to diagnose that an intramammary infection (IMI) was present. Criteria were taken from Dohoo et al. (2011), who recommended considering a quarter positive at 100 CFU/mL for all pathogens, except coagulase-negative staphylococci (CNS) for which 200 CFU/mL, are recommended if identifying as many existing infections as possible is important. A sample was considered contaminated when 3 or more pathogens were identified, except when *Staphylococcus aureus* or *Streptococcus agalactiae* were present (Olde Riekerink et al., 2008).

2.2.4 Calculation and Statistical Analyses

To simplify the examination of herd data the 1-yr period of analysis for the study was defined between April 1, 2011 and March 31, 2012. A cow was considered at risk of clinical mastitis during lactation and dry period while the herd was enrolled in the year-long study. The time at risk started with the first farm visit by researchers, the day the lactating cow entered the herd, day of calving, or 14 d after the last mastitis episode identified by the producer (Olde Riekerink et al., 2008). The risk period ended when the cow had mastitis, died, or was culled (Olde Riekerink et al., 2008). For cows with recurrent mastitis, a case was considered new and therefore included in the analysis when ≥ 14 d occurred between episodes. The incidence rate of clinical mastitis (IRCM) was expressed as the number of quarter cases per 100 cow-years at risk.

Bulk tank somatic cell count was determined monthly by the Dairy Farmers of Ontario. The geometric bulk tank somatic cell count (BTSCC) determined for each herd during the study period was used to categorize herds: Low (<150,000 cells/mL); Medium (150,000 to 250,000 cells/mL); High (>250,000) (Olde Riekerink et al., 2008).

Before performing statistical analyses, data were checked for recording errors which were corrected when identified. The associations between herd factors (farm type, housing type, and pasture use) and with IRCM were assessed using a general linear model (GLM) that incorporated all three factors and their interactions. A GLM was also used to investigate the relationship between BTSCC and IRCM. All data analysis was completed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, 2008). Statistical significance was defined as $P \leq 0.05$ and a trend was defined as $0.05 < P \leq 0.1$.

2.3 Results

Of the farms participating in the study, 70% were conventional and 30% were organic (Table 2.1). The mean daily production during the study period, from DHI test weights, was 31.5 kg/d and 20.8 kg/d for conventional (n=41 farms) and organic herds (n=15 farms), respectively. Herds utilizing some pasture for their lactating animals made up 50.8% of participating farms. Loose housing for lactating cows was used on 24 (41%) farms and tie-stall housing was used on 35 (59%) farms. Tie-stall housing for lactating animals was commonly used on conventional dairy farms (68%), whereas loose housing was more common on organic dairies (61%). There were a total of 5395 lactating cows from the 59 participating herds. The average milking herd size was 65 and ranged from

18 to 220 cows. The average milking herd size was 67 cows for organic farms (range 18 to 220) and 64 cows for conventional farms (range 22 to 220) farms.

During the study period, 883 quarter clinical mastitis samples were submitted from 705 cows. Clinical mastitis samples were submitted from 58 (98%) of participating farms (Figure 2.1). Coagulase-negative staphylococci was the most frequently isolated clinical mastitis pathogen followed by *Bacillus* spp., *Streptococcus* spp*., and Staphylococcus aureus* (Table 2.2; Figure 2.2). No-growth was detected in 22.6% of samples cultured and sample contamination rate was 6.3%. The majority of clinical mastitis samples were single culture. However, 182 samples (20.6%) were mixed, with two pathogens isolated. Mastitis severity was recorded for 91% of submitted clinical samples. Abnormal milk as the only indicator of mastitis (MSS 1) was observed in 55.5% of cases. Abnormal milk with physical changes evident in the quarter (MSS 2) was perceived by producers in 37.4% of cases and in 57 cases (7%) cows exhibited signs of abnormal milk, swollen quarter and systemic illness (MSS 3).

The mean herd IRCM was 24.7 cases per 100 cow-years are risk, and the median IRCM was 18.7 cases per 100 cow-years at risk, ranging from 0.0 to 75.0 per herd (Table 2.3; Figure 2.3). Incidence rates for clinical mastitis tended to differ by farm type (P = 0.07), with higher IRCM observed in conventional herds. The overall mean IRCM were 28.1 and 17.1 cases per 100 cow-years at risk for participating conventional herds and certified organic herds, respectively. Housing type and access to pasture were not associated with IRCM (P = 0.3 and P = 0.3, respectively). Average herd-level milk production (kg/cow/day) was not associated with IRCM (Figure 2.6).

Categorization of herds based on yearly geometric mean of their BTSCC resulted in 15 (25%), 19 (32%) and 25 (42%) herds in the low, medium, and high categories respectively (Figure 2.4). The geometric mean herd BTSCC for conventional farms was 222,000 cells/mL (SD=100) and was slightly greater for organic farms at 272,000 cells/mL (SD=88). No difference in IRCM caused by BTSCC categories was observed (P = 0.5; Figure 2.5). The specific pathogen IRCM caused by *Bacillus, Streptococcus,* CNS*, Klebsiella,* and *Staphylococcus aureus* did not vary between BTSCC categories (P > 0.05; Table 2.4). A tendency (P = 0.06) for a difference was noted in the IRCM caused by *Escherichia coli* between the low and high BTSCC categories, where low BTSCC herds had elevated *E.coli* IRCM. The IRCM caused by *Streptococcus dysgalactiae* was different between the low and medium BTSCC categories (P = 0.04). Medium BTSCC herds had lower *Streptococcus dysgalactiae* IRCM. *Escherichia coli* and *Bacillus* were the only pathogen specific IRCM that varied by farm type (P = 0.03 and P=0.02, respectively; Table 2.5). Conventional farms had a significantly greater mean IRCM caused by *Escherichia coli* and *Bacillus* compared to organic farms. Farms using loose housing had a greater (P=0.05) mean *Bacillus* IRCM compared to farms using tie-stall housing (4.6 vs. 2.4 cases per 100 cow-years). Pasture use was associated with pathogen specific IRCM, in the case of *Streptococcus* spp. (P = 0.05). The mean IRCM caused by *Streptococcus* spp. was higher in herds with access to pasture (3.1 cases per 100 cow-years) compared to herds without access to pasture for lactating cows (2.1 cases per 100 cow-years).

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2.4 Discussion

Bacteriology of clinical mastitis samples revealed CNS to be the most frequently isolated pathogen at 20.4%, which is twice as frequently as in a previous Canadian study (10.7%) that sampled clinical mastitis (Olde Riekerink et al., 2008). The frequency of CNS was similar to Reyher et al. (2012) who found CNS in 20% of samples as part of a National Cohort of Dairy Farms longitudinal quarter milk sample study. In that research milk samples were not necessarily from cases of clinical mastitis (Rehyer et al., 2012). Traditionally CNS has been considered a minor mastitis pathogen (Schukken et al., 2009). However, some species of CNS have become recognized as a predominant pathogen causing mastitis (Taponen et al., 2006; Reyher et al., 2011).

Bacillus spp. was the second most frequently isolated pathogen, accounting for 17.9% of isolates. This is contrary to previous research by Olde Riekerink et al. (2008) and Bradley et al. (2007), where *Bacillus* spp. was reported in only 2.2% and 1.5% of isolates, respectively. *Bacillus* spp. are usually considered non-pathogenic bacteria (Britten, 2012) that reside widely in the environment (Gonzalez, 1996). Increased frequency of isolation in the current study may be related to milk sampling techniques used by participating producers. Gonzalez (1996) suggested that *Bacillus* spp. might occur in samples because the teat was not properly stripped or the teat end was still humid with alcohol, at time of sample collection.

Streptococci excluding *Streptococcus agalactiae*, are usually labeled environmental streptococci. In this study Streptococci were identified into the following species: *Streptococcus dysgalactiae; Streptococcus agalactiae; Streptococcus uberis; Streptococcus canis;* and *Streptococcus* spp. *Streptococcus* spp. was the third most

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frequently isolated pathogen (11.5%); all other streptococci were found in low numbers in clinical mastitis samples (Table 2.2). As Streptococci are one of the most common etiologic agents of environmental mastitis in North America (Hogan and Smith, 2012), and 50% of environmental streptococcal infections cause clinical mastitis, it is reasonable that *Streptococcus* spp. was found at such a high rate. Sargeant et al. (1998) found a similar frequency of *Streptococcus* spp. (14%) in clinical mastitis samples in a study of 65 dairy farms located in Ontario.

Staphylococcus aureus is considered a major mastitis pathogen because of its effect on milk quality, production, and cow SCC (Keefe, 2012). *Staphylococcus aureus* was the most frequently isolated contagious mastitis pathogen in this study (9.6%), which is much lower than the 21.7% of isolates reported by Olde Riekerink et al. (2008). The reduced *Staphylococcus aureus* in this study may be related to the participating producers awareness that *Staphylococcus aureus* often persists in the udder for extended periods of time (Reyher et al., 2012) and can be a major SCC contributor (Keefe, 2012). This is relevant, since the period of study occurred just prior to the reduction in the BTSCC regulatory limit in Canada from 500,000 cells/mL to 400,000 cells/mL. Considering that 42% of participating herds were in the high BTSCC group, producers might have been actively trying to reduce BTSCC, which may have included strategic culling of *Staphylococcus aureus* infected cows.

No-growth was detected in 22.6% of samples, which is lower (43.9%; Olde Riekerink et al., 2008) and higher (17.6%; Sargent et al., 1998) than previous reports. Negative cultures could have resulted from the freezing of milk samples (Schukken et al., 1989), spontaneous bacterial cure, the presence of too few bacteria for culture (Zorah et

al., 1993), or from samples that truly did not contain a pathogen, which could be related

to producer case identification. The contamination rate of clinical mastitis samples was

lower in this study than previous research (Sargent et al., 1998; Olde Riekerink et al.,

2008), suggesting that farmers collecting samples performed proper sampling techniques.

Contaminated samples came from 36% of participating farms (n=21). The greatest

proportion of contaminated samples came from one conventional loose housing farm,

which contributed 16% of the overall contaminated samples.

The mean herd IRCM determined in this study (24.7 cases per 100 cow-years)

was similar to that of other Canadian research: 26.3 cases per 100 cow-years (Thompson-

Crispi et al., 2013); 23.0 cases per 100 cow-years (Olde Riekerink et al., 2008); 19.8

lactational incidence risk (Sargent et al., 1998); and 21.8 per 100 lactations (McLaren et

al., 2006). Sargeant et al. (1998) determined IRCM using only full 305-day lactations. It

has been suggested that the criteria used by Sargeant et al. (1998) could have

underestimated true IRCM as cows with clinical mastitis are more likely to be culled

before completing a lactation (Olde Riekerink et al., 2008). The slightly lower IRCM

reported by McLaren et al. (2006) could be related to inclusion of data from farms with as

few as 3 mo of disease reporting; therefore a clear representation of yearly disease

incidence may have been under evaluated. As previously reported (Barkema, 1998;

Sargent et al., 1998; Olde Riekerink et al., 2008), considerable variation in IRCM

between herds was observed (Figure 2.3).

Clinical mastitis sample collection was entirely dependent on producer

willingness to collect milk samples, and the selection criteria they employed (Barkema et

al., 1998). Producers may under-report cases of clinical mastitis for a variety of reasons,

including apathy towards disease control or lack of time (Bartlett et al., 2001). Strategies used to encourage producer collection involved farm visits and/or phone calls at least once every 8 wks, as well as reporting of milk sample bacteriology results to the producer as frequently as possible throughout the study period. As reporting bacterial culture results was often delayed, it is unlikely that producers over reported to obtain culture information for treatment decisions (Sargent et al., 1998). Monthly sample submission during the study period ranged from 32 (November, 2011) to 108 (May, 2011); the mean sample submission per month was 70. It has been observed that organic producers less often report cases of clinical mastitis (Berry and Hillerton, 2002; Ruegg, 2009). Sato et al. (2005) stated that farmers generally record or remember diseases treatments, rather than the episodes of clinical disease itself. Decreased reporting in organic herds could, thus, be related to reduced antibiotic use (Ruegg, 2009).

A clear definition of clinical mastitis was stated and reviewed with all producers at the start of the study in an attempt to standardize case identification. However, producer detection bias could have occurred. It has been suggested that producers may be more likely to sample cows with severe clinical mastitis (Olde Riekerink et al., 2008), which have extremely obvious clinical signs. However, this situation may not be the case in the current study where 55.5% of recorded MSS were in the lowest severity category. Producers who fore-strip and inspect milk closely might detect more clinical mastitis (Barkema et al., 1999; Olde Riekerink et al., 2008). However, Lam et al. (1993) found that differences did exist in visual scoring of clinical mastitis between producers, but the reporting of clinical mastitis by producers was not negatively impacted. As a result of the

findings by Lam et al. (1993), Sargent et al. (1998) considered that the variability in IRCM among herds represented a true difference in herd clinical mastitis incidence.

On-farm clinical mastitis research has used herd health records, producer sampling, retrospective producer recall, and national health recording systems to identify IRCM (Barkema et al., 1998; Bartlett et al., 2001; Sato et al., 2005). The current study relied solely on producer submitted clinical mastitis samples and asked producers to sample every quarter case of clinical mastitis prior to treatment. There was a large range in the number of clinical mastitis samples submitted by participating herds. This finding could reflect a true range in farm IRCM or could be due to the failure of some producers to reliably report disease occurrence. Individual cow SCC has been used to predict clinical mastitis cases (Green et al., 2004; de Haas et al., 2004). To help confirm accurate producer reporting, an investigation using SCC might have been useful; however, was not conducted at this time.

No Canadian studies have reported the IRCM on organic dairies. Researchers in other countries have investigated the IRCM in organic herds (Hardeng and Edge, 2001; Sato et al., 2005; Fall et al., 2008); in Wisconsin, USA, Sato et al. (2005) conducted an observational study to compare production and management on geographically paired organic and conventional dairy farms. They found that the BTSCC (262,000 and 285,000 cells/mL) and IRCM (28 and 32 cases per 100 cow-years at risk) were not different between organic and conventional management systems. Fall et al. (2008) compared udder health of organically and conventionally managed cows on a Swedish dairy research farm to eliminate the possible confounder of general management when comparing between the different systems. The 12-yr longitudinal project which used

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information obtained from the Swedish official milk recording scheme found no difference in udder health between the two systems when considering mean geometric SCC, high monthly SCC tests (>200,000cells/mL; incidence rate ratio = 0.65), and veterinary treated cases of clinical mastitis (OR = 0.43) (Fall et al., 2008). The Norwegian Dairy Herd Recording system was used by Hardeng and Edge (2001) to investigate differences in disease incidence between organic and conventional herds matched on size, and region. Hardeng and Edge (2001) found lower treatment rates for clinical mastitis (OR = 0.38), and a higher overall geometric mean BTSCC, in organic herds. A suggested explanation for the lower incidence of veterinary treated mastitis was the inconsistent use of medication within organic farming; thus, each case of mastitis would not necessarily be treated by a veterinarian and few cases would be recorded in the national database (Hardeng and Edge, 2001).

While there is much clinical mastitis research in organic management systems outside of Canada, Ruegg (2009) cautions against comparison of studies from different countries as the organic standards producers are required to maintain differ widely. As a result, research directly comparing udder health in organic and conventional managed herds has yielded varying results. In organic systems, researchers have reported higher (Bennedsgaard et al., 2003; Hamilton et al., 2006), lower (Nauta et al., 2006; Roesch et al., 2007) and no difference (Sato et al., 2005; Fall et al., 2008; Haskell et al., 2009) in udder health when compared to conventionally managed herds. These reports have looked at a variety of different measures including individual cow SCC, BTSCC, IRCM, and California Mastitis Test. In the current study, IRCM tended to be higher in conventional herds compared to organic herds. Organic systems have been associated

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with lower milk production (as also noted in this study) and reduced use of concentrates (Hardeng and Edge, 2001). Canadian organic dairy producers are specifically required to have at least 60% of DM in daily rations consist of hay, fresh/dried fodder or silage (Section 6.4.3 of the Canadian Organic Production Systems General Principles and Management Standards, 2011). As well, organic producers have suggested that the reduced production stress on organic cows may result in those animals maintaining higher immune function (Sato et al., 2005). Although it is generally assumed that animals have better immune responses under organic conditions, this assumption has not been proven (Kijlstra and Eijck, 2006). The factors mentioned above may support the lower IRCM found in organic herds. High production has been associated with greater mastitis incidence (Schukken et al., 1990; Grohn et al., 1995), and Nyman et al. (2007) found that lower levels of concentrates were fed in herds with low incidence rates of veterinary treated clinical mastitis. Reduced rates of clinical mastitis may be related to the organic management system; however, it must be noted that there are a wide variety of mastitis risk factors that are not specific to organic production, including: animal age, production level, genetics, environmental conditions, nutrition and housing (Ruegg, 2009). Further investigation is needed to identify the significant management factors that impact clinical mastitis incidence on these farms.

Housing and access to pasture for lactating animals had no association with the overall mean IRCM for participating herds. Where housing and pasture had an association was with pathogen specific IRCM. Farms using loose housing had a greater mean *Bacillus* IRCM. *Bacillus* spp. can grow in soil deposits (Britten, 2012) and are widely distributed in the environment, soil, water, dust, air, feces and on vegetation

(Gonzalez, 1996). The higher IRCM noted in loose housing implies higher cow exposure to this pathogen in varied areas of their housing. Pasture is commonly considered to reduce mastitis pathogen exposure (Hogan and Smith, 2012). However, this association does not hold true for *Streptococcus* spp. in the current study, in which IRCM was found to be higher in herds with pasture access. Environmental pathogens are typically found where a cow rests (Ferguson et al., 2007). As barren soil and wet conditions may contribute to bacterial populations in the environment (Hogan and Smith, 2012), our results suggest that the pasture lying environment may have not been ideal and actually increased pathogen contact.

Similarly to previous research, mean IRCM did not vary between BTSCC categories. However, pathogen specific IRCM was associated with BTSCC (Barkema et al., 1998; 1999). Barkema et al. (1999) pointed out that these relationships are not necessarily causal. Researchers (Barkema et al., 1999; Olde Riekerink et al., 2008) have reported higher *E.coli* IRCM in herds with low BTSCC. This research had similar results, with a tendency for low BTSCC category farms to have a higher incidence of *E.coli* IRCM compared to high BTSCC herds. The lower incidence of *Streptococcus dysgalactiae* IRCM for those herds in the medium BTSCC category compared to the low BTSCC group is consistent with results obtained by Barkema et al. (1998). Specific management factors have been related to incidence of *E.coli* in low BTSCC herds and *Streptococcus dysgalactiae* in higher BTSCC (Barkema et al., 1999). Researchers suggested that post-milking teat disinfection reduces minor pathogens enhancing the opportunity for major pathogens such as *E.coli* (Schukken et al., 1989). Barkema et al. (1999) commented that post-milking teat disinfection was associated with a decreased

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IRCM due to *Streptococcus dysgalactiae* in high BTSCC. Investigation into herd management practices consistent within each BTSCC category is needed to identify factors impacting the variability of pathogen-specific IRCM between BTSCC categories.

2.5 Conclusions

The mean herd IRCM on dairy farms in Southern Ontario was 24.7 cases per 100 cow-years at risk. Mean IRCM tended to vary by management system, with 28.1 and 17.1 cases per 100 cow-years at risk in conventional and organic herds, respectively. Housing system and access to pasture for lactating cows, as well as BTSCC category was not associated with overall IRCM. However, these factors were associated with pathogen-specific IRCM. Reduced rates of clinical mastitis may be related to the organic management system. Yet, it must be noted that there are a wide variety of mastitis risk factors that are not specific to organic production. Further investigation is needed to identify the significant management factors that impact IRCM on organic farms.

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Cluster, a part of the Canadian Agri-Science Clusters Initiative of Agriculture and Agri-Food Canada's Growing Forward Policy Framework.

Table 2.1. Distribution of participating farms

| Housing type | Number of farms | Conventional (n=41) | | Organic (n=18) |
| | | Pasture use | | |
		Yes	No	
Tie-stall	35	9	19	7
Free-stall	18	3	10	5
Pack	6	0	0	6
Total	59	12	29	18

Table 2.2. Distribution of mastitis pathogens in 883 submitted clinical mastitis samples

Pathogen	Frequency	Percentage of samples (%)	Percentage of isolates (%)
Major			
Staphylococcus aureus	78	8.8	9.6
Streptococcus dysgalactiae	21	2.4	2.6
Streptococcus uberis	4	0.4	0.5
Streptococcus agalactiae	1	0.1	0.1
Escherichia coli	75	8.5	9.2
Klebsiella spp	20	2.3	2.5
Enterobacter spp	28	3.2	3.5
Minor			
Coagulase-negative staphylococci	165	18.7	20.4
Bacillus spp	145	16.4	17.9
Streptococcus spp	93	10.5	11.5
Corynebacterium spp	30	3.4	3.7
Enterococcus spp	29	3.3	3.6
Other Gram-negative cocci	21	2.4	2.6
Trueperella pyogenes	19	2.2	2.3
Staphylococcus hyicus	15	1.7	1.9
Yeast	14	1.6	1.7
Other Gram-negative rods	14	1.6	1.7
Pseudomonas spp	12	1.4	1.5
Proteus spp	6	0.7	0.7
Streptococcus canis	6	0.7	0.7
Fungus	4	0.4	0.5
non-Staphylococcus aureus coagulase positive Staphylococcus spp	3	0.3	0.4
Serratia spp	2	0.2	0.2
Rhodococcus spp	1	0.1	0.1
Oxidase positive Gram negative rods (Alpha & Gamma Hemolysis)	1	0.1	0.1
Alpha hemolytic Gram-negative cocci	1	0.1	0.1
Mixed culture[1]	182	20.6	-
Contaminated[2]	56	6.3	-
No growth	200	22.6	-

[1] Sample with two pathogens identified on culture

[2] Sample with 3 or more pathogens identified on culture

Table 2.3. Distribution of participating herds and incidence rate of clinical mastitis

Farm type	Number of herds	Number of quarter CM[1] samples submitted	Total cow years at risk[2]	Mean IRCM[3]	95% CI	Mean geo-BTSCC[4]
Organic loose housing	11	119	925	13.6	2.0-25.1	306
Organic tie housing	7	68	330	22.7	8.2-37.1	218
Conventional loose housing w pasture	3	109	338	32.5	10.3-54.4	411
Conventional loose housing w/o pasture	10	239	1010	23.1	11.0-35.2	154
Conventional tie housing w pasture	9	92	308	32.8	20.1-45.6	225
Conventional tie housing w/o pasture	19	256	1051	27.7	19.0-36.5	226
Organic	18	187	1255	17.1	8.2-25.9	271
Conventional	41	696	2707	28.1	22.1-33.9	222
All farms	59	883	3962	24.7	-	-

[1] CM = Clinical mastitis
[2] Calculated on a 365 d basis for each cow
[3] IRCM = Incidence rate of clinical mastitis per 100 cow-years at risk
[4] Geo-BTSCC = Geometric mean bulk tank somatic cell count (x 1000 cells/mL)

Table 2.4. Least-squares mean incidence rate of clinical mastitis (IRCM per 100 cow-years at risk) for select pathogens within bulk tank somatic cell count (BTSCC)

| Pathogen	BTSCC (x1000 cells/mL)		
	<150 (n=15)	151-250 (n=19)	>250 (n=25)
CNS	4.3	3.2	3.9
Bacillus	4.9	2.3	3.2
Streptococcus	2.6	1.4	3.5
Staphylococcus aureus	2.3	3.1	1.7
Escherichia coli	3.7[a]	1.8	1.2[a]
Streptococcus dysgalactiae	1.2[b]	0.2[b]	0.6
Klebsiella	0.4	0.3	0.6
No-growth	6.5	7.4	4.8
Overall mean IRCM	29.2	22.7	23.6

[a] IRCM on the same row having a common superscript differ (P=0.06)
[b] IRCM on the same row having a common superscript differ (P=0.04)

Table 2.5. Least-squares mean incidence rate of clinical mastitis (IRCM per 100 cow-years at risk) by farm type

| Pathogen	Conventional	Organic
CNS	4.4	1.7
Bacillus	5.1[a]	0.18[a]
Streptococcus	2.9	1.5
Staphylococcus aureus	2.4	1.8
Escherichia coli	3.0[a]	0.0[a]
Streptococcus dysgalactiae	0.5	0.8
Klebsiella	0.4	0.4
No-growth	6.5	4.3
Overall mean	28.1	17.1

[a] IRCM on the same row having a common superscript differ (P<0.03)
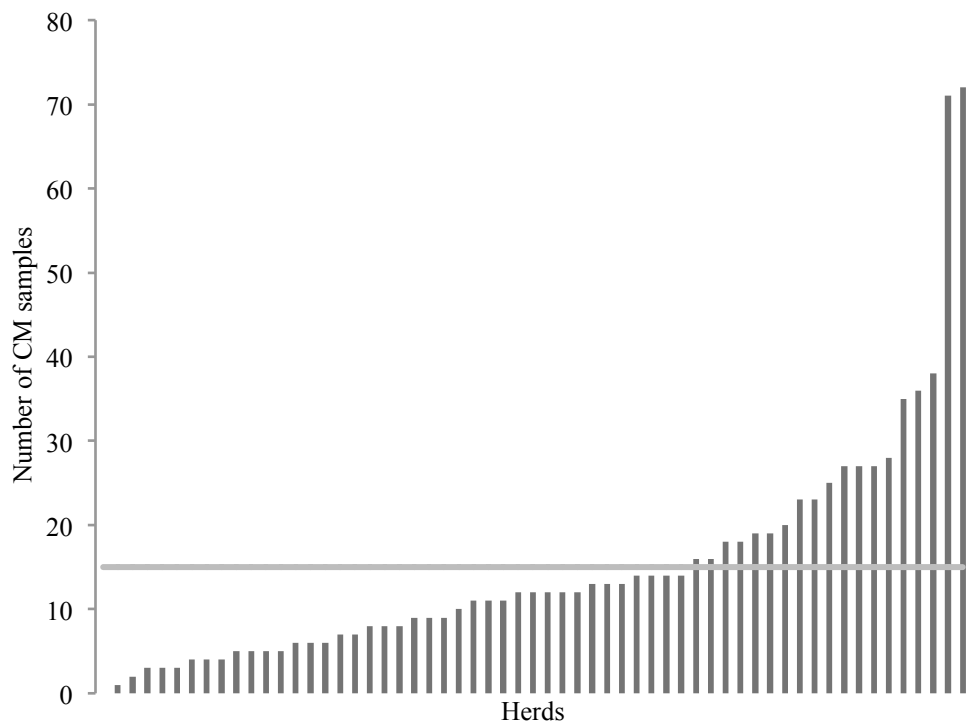
Figure 2.1. Distribution of quarter clinical mastitis (CM) samples submitted by farm
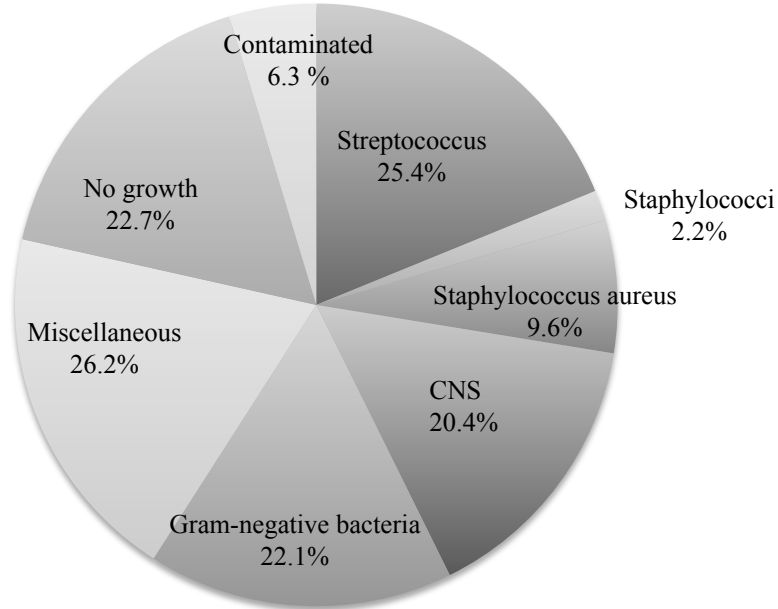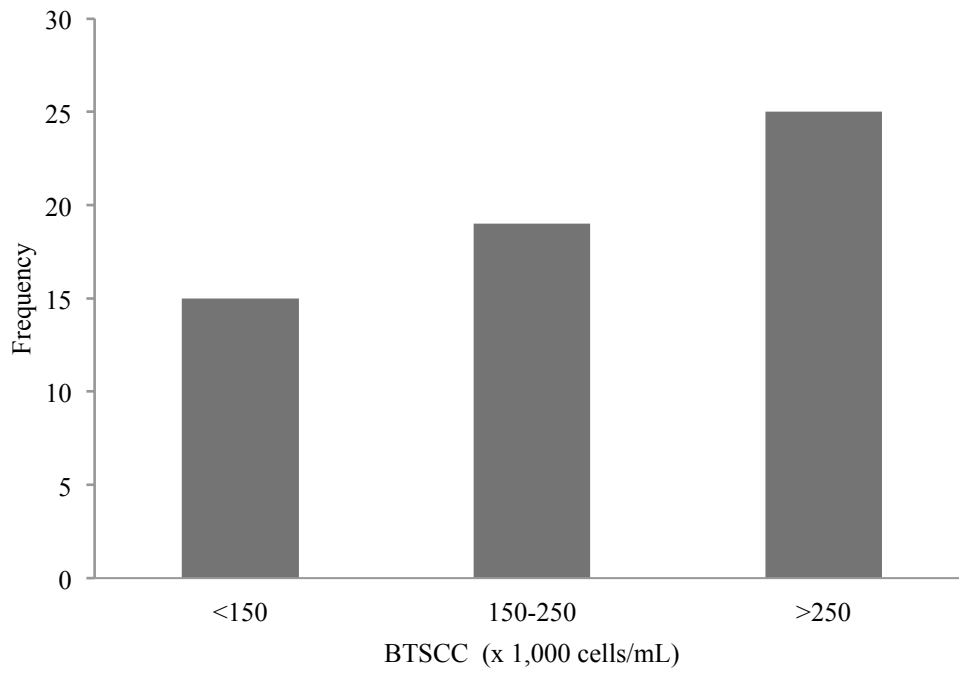
Figure 2.2. Distribution of quarter clinical mastitis pathogens by percentage of isolates *(*Streptococcus: *Streptococcus spp., Enterococcus spp., Streptococcus dysgalactiae, Streptococcus canis, Sreptococcus uberis, Streptococcus agalactiae;* Staphylococci: *Staphylococcis hyicus, non-Staphylococcus aureus coagulase positive Staphylococcus spp;* Gram-negative bacteria: *Escherichia coli, Enterobacter spp., Other Gram-negative cocci, Klebsiella spp., Other Gram-negative rods, Pseudomonas spp., Proteus spp., Serratia spp., Oxidase positive Gram-negative rods, Alpha-hemolytic Gram-negative cocci;* Miscellaneous: *Bacillus, Corynebacterium spp., Trueperella pyogenes, Yeast, Fungus, Rhodococcus spp.)*
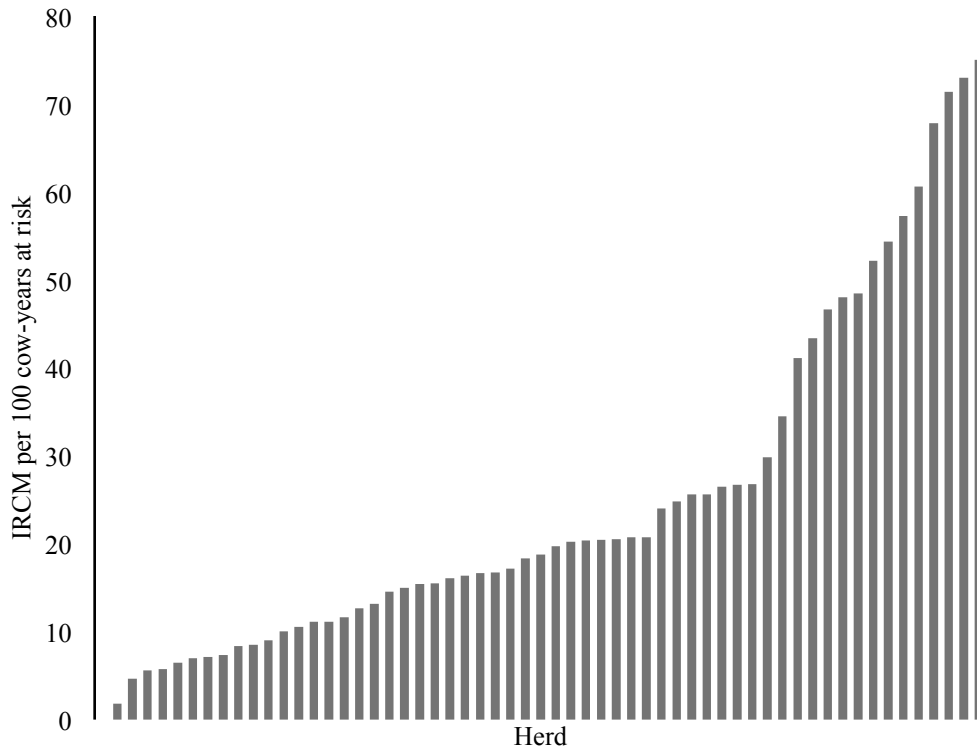
Figure 2.3. Incidence rate of clinical mastitis (IRCM) in the 59 herds that participated in the study
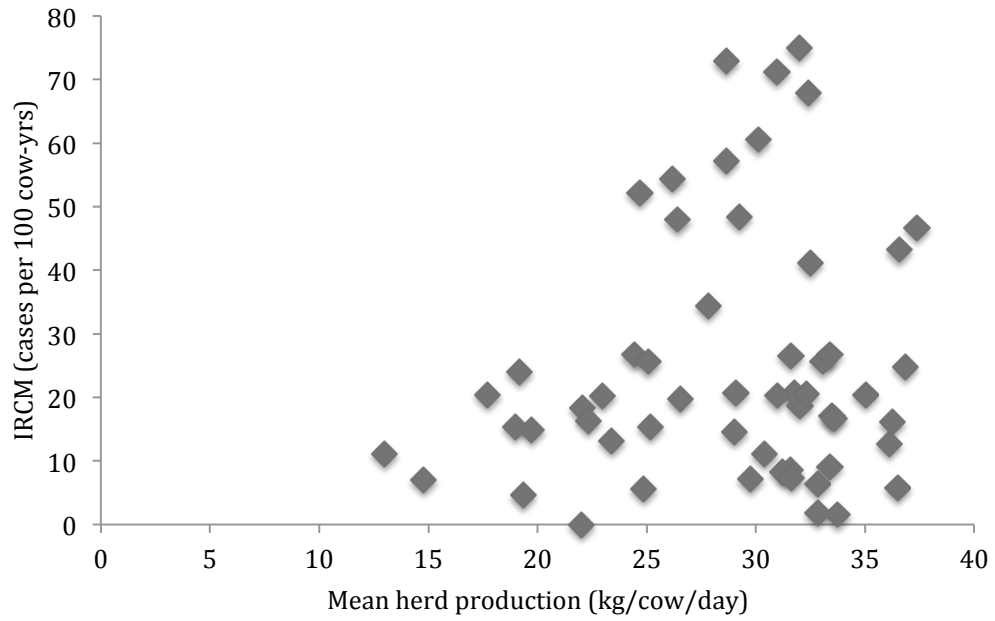
Figure 2.4. Distribution of herds by bulk tank somatic cell count (BTSCC) category based on geometric mean of monthly BTSCC during the study period
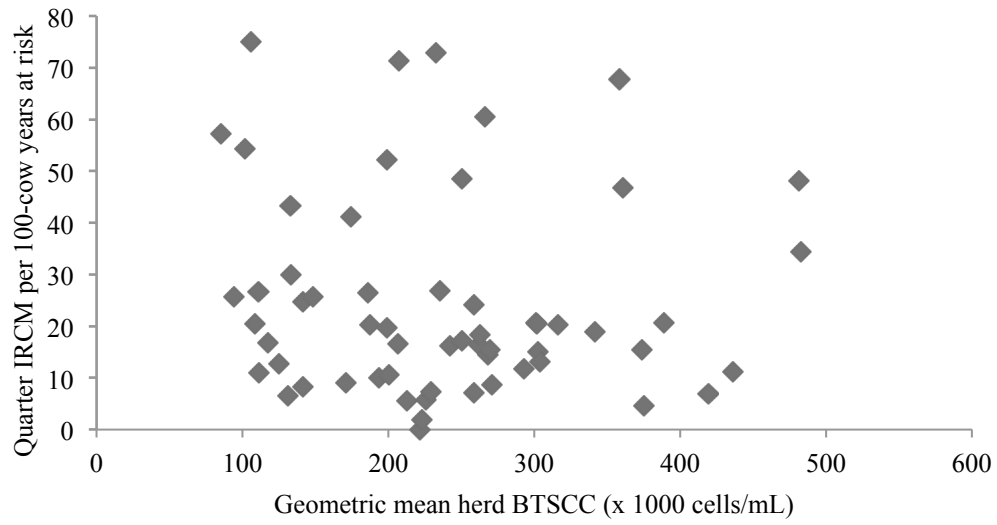
Figure 2.5. Incidence rate of clinical mastitis (IRCM) vs. geometric mean bulk tank somatic cell count (BTSCC)

Figure 2.6. Incidence rate of clinical mastitis (IRCM) vs. mean herd production

CHAPTER 3: GENERAL DISCUSSION

3.1 Important Findings

Organic milk production has doubled in Canada from 2005 to 2010 (Agriculture & Agri-Food Canada, 2012). Consumers are considered a major driving force for the growth of the organic industry and are motivated to purchase for a variety of reasons including decreased chemical residues and perceived product quality (McEachern and McClean, 2002; Pearson et al., 2010). Perceived higher animal welfare is another reason consumer purchase organic (Pearson et al., 2010). One aspect impacting dairy cow welfare is clinical mastitis. Clinical mastitis can be a severe and painful disease that negatively impacts cattle welfare and it is therefore important to decrease the clinical incidence of this disease (Schukken et al., 2003). To date, no previous Canadian mastitis research has been specifically focused on determining incidence rate or predominant pathogen types of clinical mastitis within organic dairy herds. The research described in this thesis was conducted to understand the incidence rates and predominant pathogen types of clinical mastitis on organic dairy farms in Southern Ontario, Canada.

The mean herd IRCM determined was 24.7 cases per 100 cow-years at risk, which is comparable to previous Canadian research (Olde Riekerink et al., 2008; Thompson-Crispi et al. 2013). Incidence rates for clinical mastitis tended to be lower in the observed organic herds. The overall mean IRCM for participating conventional herds was 28.1 cases per 100 cow-years are risk and mean IRCM was 17.1 cases per 100 cow-years at risk for certified organic herds. Research often reports lower IRCM on organic farms when compared to conventional (Hamilton et al., 2002; Hamilton et al., 2006) which may represent a truly reduced incidence of clinical mastitis in organic production systems or

may be deceptively low due to lack of reporting by organic producers (Berry and Hillerton, 2002; Ruegg, 2009). Housing type and access to pasture did not impact overall IRCM suggesting the overall IRCM is consistent notwithstanding the environment in which cows are housed.

Conventional farms had a significantly greater mean IRCM for *Escherichia coli* and *Bacillus* compared to organic farms. These results indicate that conventional farm management practices increase cow exposure to these environmental mastitis pathogens. Further investigation into conventional herd management practices is needed to identify specific factors implicated in this increased exposure.

Farms using loose housing had nearly twice the *Bacillus* IRCM compared to farms using tie stall housing suggesting that a loose housing environment increases cow exposure to *Bacillus*. Pasture use did not affect pathogen specific IRCM, except in the case of *Streptococcus* spp*.*, which was 50% higher in herds with access to pasture compared to herds without access to pasture for lactating cows. Pasture is commonly considered to reduce mastitis pathogen exposure (Hogan and Smith, 2012). However, in the case of *Streptococcus* spp. reduced exposure may not hold true suggesting that pasture should not be excluded when considering areas of risk for mastitis pathogens exposure in cows.

There was no difference in IRCM between herds of different BTSCC categories. Low BTSCC herds tended to have elevated *E.coli* IRCM compared to high BTSCC herds. Medium BTSCC herds had lower *Streptococcus dysgalactiae* IRCM than low BTSCC herds. These results are consistent with previous research (Barkema et al., 1999;

Olde Riekerink et al., 2008), suggests that although herds may manage their BTSCC well, they are still at risk of clinical mastitis due to major pathogens.

3.2 Future Research

This research is a positive first step towards understanding the IRCM found on organic dairy farms in Canada; however, considerably more investigation is need to establish a comprehensive view. Future research should include a larger number of organic dairy producers. The current study included only ~9% of Canadian organic dairy producers and was limited to one geographic area. Incidence rates and predominant pathogens may be altered with the inclusion of a larger number of producers from a wider geographic area. The inclusion of a greater number of producers would also facilitate a more complete understanding of management factors employed by organic producers.

The current study employed a producer questionnaire to investigate how housing and management factors may have influenced outcomes; this analysis is still forthcoming. It is particularly important to understand management practices of organic producers as research in other countries has identified difference in organic management practices from conventional norms either as a result of regulatory restrictions or philosophical differences (Tikofsky, 2005). Differing management also impacts collection of data to determine IRCM (Pol and Ruegg, 2007), as well as management strategies for prevention and treatment of clinical mastitis.

The current study used only producer-collected samples of clinical mastitis to identify incidence rates. Future research should include collected samples in concert with producer records in an attempt to obtain a more complete account of clinical cases.

Investigation of individual cow SCC in a herd should also occur to verify producer reporting of clinical mastitis cases; high composite SCC has been associated with subsequent cases of clinical mastitis (van den Borne, 2011).

Given some of the pathogen specific findings, bacteriological culture of the lying surfaces of indoor housing and at pasture should be considered to further investigate the predominant environmental pathogens responsible for clinical mastitis cases. It would be most appropriate to culture the lying surfaces of cows as apposed to other areas of housing as environmental pathogens are typically found where a cow rests (Ferguson et al., 2007).

A final consideration for future research would be to obtain baseline herd culture results upon herds entering the study. By performing herd bacteriology, researchers could establish an understanding of common pathogens in a herd, and existing mastitis infections. This information could be used to identify problem herds.

3.3 Implications

Overall, this research is important to establish a baseline understanding of IRCM and pathogens responsible for CM to have effective monitoring of future changes within the organic dairy industry. Understanding causative mastitis agents allows appropriate management strategies to be employed (Cressier and Bissonnette, 2011) and supports the implementation of preventative strategies which help satisfy the organic philosophy of promoting health and reducing use of synthetic therapeutics (Tikofsky, 2005). This research could, thus, serve as a sentinel for IRCM within the Canadian organic dairy system and serve to stimulate further research in this area.

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