Chapter 17

Bovine Mastitis Pathogens: Prevalence and Effects on Somatic Cell Count

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Additional information is available at the end of the chapter

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1. Introduction

Bovine mastitis is the most prevalent and costly disease, affecting dairy farms worldwide. Economic losses associated with mastitis derive mainly from a decrease in milk production and to a lesser extent, from the culling of chronically infected cows, cost of veterinary treatment, and penalties on milk quality (Seegers *et al*., 2003). Mastitis is caused by a wide spectrum of pathogenic agents that penetrate the teat canal and multiply in the udder cistern. The majority of mastitis cases are produced by a relatively small group of bacteria, including *Staphylococcus aureus*, *Streptococcus uberis, Mycoplasma spp* and *Escherichia coli* (Calvinho & Tirante, 2005). Bovine mastitis is characterized by inflammation of the mammary gland. The inflammation severity depends on the causative agent and the host response (Bannerman *et al*., 2004; Barkema *et al*., 2006; Burvenich *et al*., 2003; Petzl *et al*., 2008). Resident and recruited cells together play an essential role in immediate defense against local infection (Rainard & Riollet, 2006). Extensive neutrophil recruitment from the circulation to the lumen of the mammary gland is a hallmark of the early immune response to mammary infection (Thomas *et al*., 1994; Sordillo & Streicher, 2002; Oviedo-Boyso *et al*., 2007). When designing mastitis-prevention and control programs, it is worthy to take on account the adoption level of mastitis-prevention management practices and control programs as well as the etiology of the intramammary infections (IMI), the herd-level prevalence of contagious mastitis pathogens, and the general factors that influence milk production.

2. Mastitis pathogens agents

Staphylococcus aureus

Staphylococcus aureus (*S. aureus*) colonize the nipple skin, advance through the mammary gland canal into the gland. The IMI with *S. aureus* predominantly cause subclinical mastitis resulting in a chronic infection lingering lifelong (Bannerman *et al*., 2004; Riollet *et al*., 2000; Yang *et al*.,

2008). During the infection's early stages, the mild damage may be reverted but *S. aureus* infections, in its peracute mastitis presentation generates gangrene and severe tissue damage. In comparison with *Streptococcus agalactiae*, *S. aureus* is more difficult to be eradicated. *S. aureus* infections cause a 45% decrease on milk production per quarter, reflected as a 15% per infected animal (NMC, 1999). The chronic, subclinical infections account for approximately 80% of mastitis related costs, due to reduced milk yield and product quality (Shim *et al*., 2004). In practice, an elevated somatic cell count (SCC), over 300, 000 to 500, 000 cells/ml, indicates high prevalence of infected glands with *S. aureus* in a herd (NMC, 1999).

Streptococcus agalactiae

Streptococcus agalactiae (*S. agalactiae*) causes contagious mastitis, an obligated pathogen of the mammary gland, which is transmitted directly among cows during milking (NMC, 1999). *S. agalactiae* infects the gland cistern and ducts of the mammary gland causing irritation, swelling and subclinical mastitis. The infected cow shows mere clinical signs without abnormalities drawn in milk. However, low production rates and high SCC are usually registered. *S. agalactiae* infections are related to Bulk-tank milk figures around a 1,000, 000 cells/ml on SCC or higher. Currently, these figures are rarely seen because the control measures and milking management had been improving along with better antibiotic treatment (Hillerton & Berry, 2003).

Globally *S. agalactiae* is a low prevalence pathogen. In Canadian bulk milk, its prevalence ranged between 6% in Alberta (Schoonderwoerd *et al*., 1993), and 43% in Québec (Guillemette *et al*., 1992). In the Prince Edward Island, Keefe *et al*., (1997) determined a herd prevalence of 18%. Furthermore, a study recently performed in Canada (Richard G.M. *et al*., 2010) demonstrated the low prevalence of *S. agalactiae* at 4.4% and in Argentina, in the last 25 years, the mastitis prevalence due to *S. agalactiae* has been 0.3% in the four quarters before-delivery (Calvinho *et al*., 2001).

***Mycoplasma* spp**

Mycoplasma spp are highly contagious microorganisms, but less common than *S. agalactiae* and *S. aureus.* Nevertheless, *Mycoplasma* spp damage the secretory tissue, induce the gland fibrosis, abscesses and the lymphatic nodules fibrosis (NMC, 1999). Animals from all ages are susceptible, as well as at any time during lactation. Those in early lactation are more susceptible to *Mycoplasma* infection and it can be isolated from high production animals without signology.

Mycoplasmosis is frequently related to the mastitis outbreak onsets, to the introduction of new animals to a herd, to previous respiratory or articular disease, and to herds with unresponsive mastitis to antibiotic treatment. When at least the recurrent mastitis, a non-signs illness and an unresponsive treatment are observed, a mycoplasma infection is suspected.

Mycoplasma infection prevalence at the herd-level is estimated by *Mycoplasma* culture from Bulk Tank Milk (BTM) and has been suggested to be between 1 and 8%in the USA (Fox LK., *et al*., 2005). These monitoring of mycoplasma-mastitis performed through BTM cultures assumes that the appearance of a *Mycoplasma* sp in it indicates that there is at least one cow in the herd affected with mycoplasma and that environmental contamination of the bulk tank by

mycoplasma is unlikely, hence a false positive result is discarded. The speciation of mycoplasma mastitis pathogens requires secondary tests, usually only carried out by specialized laboratories from colonies presumptively identified as *Mycoplasma* spp and with specific end point PCR for *M. alkalescens, M. bovigenitalium, M. bovirhinis, M. californicum, M. canadense* and *M. bovis* (Hirose *et al*., 2001; Kirk JH. *et al*., 1997) applied to determine the genus and specie prevalence from BTM samples collected monthly between 1989 and 1995 from 267 dairy herds. From these *M. bovis, M. canadense, M. californicum , M. bovigenitalium, M. alkalescens*, were retrieved from 209 (78.2%) dairies and they had been identified and reported as potentially pathogenic *Mycoplasma* organisms. Further studies, in the herd level such as, Fox *et al*., (2003) and the Nothwest Dairy Association (NDA), processed milk from 463 herds concluding 93 herds diagnosed as mycoplasma-positive from BTM. *Mycoplasma* was more likely to be present in samples from herds shipping higher milk amounts, therefore mycoplasma is indirectly related to the herd size and the larger the herds are, the higher *mycoplasma* caused mastitis prevalence will be. From the same study, a year later, *Mycoplasma* spp were not detected in any herd. These finding suggested that Mycoplasma caused mastitis can be controlled and eliminated from herds. This observation is supported by the studies done by, Brown *et al*. (1990), who reported that an outbreak of *Mycoplasma bovis* mastitis was controlled by an intensive identification scheme to find infected cows, culling the unproductive ones, and segregating and milking the left under a milking time hygiene procedure, also Bicknell *et al*. (1983) reported similar findings with intensive identification schemes to determine cows with *Mycoplasma bovis* mastitis and successfully managed with segregation and culling. Similar findings were reported by Mackie *et al*. (2000) specifically for *M. californicum* and *M. canadense*. The exception was reported by Jackson and Boughton (1991) who observed that segregation and culling were not necessarily required for controlling a *M. bovigenitalium* outbreak.

Coagulase-negative *Staphylococci* (CNS)

Coagulase-negative *Staphylococci* (CNS) are considered opportunistic mastitis pathogens, resident colonizers on the teat skin, rarely causing clinical mastitis (Hogan *et al*., 1987) and are frequently not reported in mastitis studies (Bramley & Dodd, 1984). However, CNS are isolated from cases of subclinical and clinical mastitis and as the cause of IMI in lactating cattle with subclinical prevalence of 31.1% at prepartum and 27.9% postpartum (Hogan, 1997; Fox , 2009). Moreover, CNS are the most frequently isolated pathogens from mastitis in heifers. This bacteria group comprises more than 50 species and subspecies (Pyöräla S. *et al.,* 2008). Coagulase-negative *Staphylococcus* species differ from each other in antimicrobial susceptibility, virulence factors and host response to infection (Birgersson *et al*., 1992; Devriese *et al*., 2002; Taponen S. *et al*., 2009). Thus, identification of species may be relevant for epidemiological surveys, the assessment of their pathogenic significance and for developing specific management practices to prevent mastitis. Perhaps it could be worthy to study them as individual species. There are many differences regarding the pathogenicity of different species of CNS that are studied with molecular diagnostic techniques (Zadoks & Schukken, 2006).

The most commonly isolated species of CNS from bovine mastitis are *Staphylococcus chromogenes, Staphylococcus epidermitis*, *Staphylococcus hyicus* and *Staphylococcus simulans.* Prevalence studies have demonstrated that CNS are the bacteria group most frequently isolated

from milk samples with high SCC (Pitkälä *et al*., 2004; Bradley *et al*., 2007; Piepers *et al*., 2007; Sampimon *et al*., 2009). In mammary quarter infection prevalence ranges between 28.9–74.6% prepartum, and 12.3–45.5% at calving. CNS are the most prevalent cause of subclinical IMI in heifers and coagulase-positive *Staphylococci* (CPS) are the second most prevalent pathogens, while in other studies the environmental mastitis pathogens are more prevalent. Generally, the pathogens that cause mastitis in heifers are the same as those that cause infections in older cows. The risk factors for subclinical mastitis appear to be dependent on the season, herd location, and trimester of pregnancy; all suggesting that management has great impact in the prepartum disease control. Regarding clinical mastitis, the most prevalent mastitis pathogen has been reported to be CNS as well as CPS and environmental mastitis pathogens. Heifers are at a higher risk for clinical mastitis during the periparturient period including those related to diet, intrinsic mammary gland factors such as swelling and milk leaking, and factors associated with management changes and the heifer's introduction the milking herd (Fox, 2009).

The prevalence of IMI with CNS has been increasing in North America, Europe and Latin America (Calvinho *et al*., 2001, Jánosi1 & Baltay, 2004; Sampimon *et al*., 2009; Pantoja, *et al*., 2009) (Table 1 and Table 2). CNS are the most frequently isolated pathogen group from IMI in The Netherlands, estimated as 10.8% at the quarter level and 34.4% at the cow level. Fourteen species of CNS were identified and the most relevant were *Staphylococcus chromogenes* (30.3%) *Staphylococcus epidermidis* (12.9%) and *Staphylococcus capitis* (11.0%) and prevalence of CNS IMI was higher in heifers than in older cows. Geometric mean quarter SCC of CNS-positive quarters was 109,000 cells/ml, which was approximately twice as high as culture-negative quarters. Quarters infected with *S. chromogenes*, *S. capitis* and *Staphylococcus xylosus* had a higher SCC ($P < 0.05$) than culture negative quarters, while quarters that were culture-positive for *S. epidermidis* and *Staphylococcus hyicus* tended to higher SCC than culture-negative quarters. An increased prevalence of CNS-IMI is associated with the herd-level variables such as a taped source of drinking water, single dry-cows housing, monthly SCC measure, veterinary udder health monitoring, outdoors season pasturing, percentage of milk contaminated stalls, and bulk milk SCC (BMSCC) > 250,000 cells/ml. Currently the prevalence of CNS-IMI is already high in heifers around their first calving (Borm *et al*., 2006), the lower prevalence of CNS in multiparous cows may be explained by the fact that in the 80% of the farms included in this study, the practice of antibiotic dry off and post-milking teat disinfection applied twice a day during lactation was used. Also pasturing during the outdoor season was associated with an increased prevalence of CNS-IMI, and the summer period is related to active flies, especially the horn fly *Haematobia irritans* which can transmit *S. aureus* (Owens *et al*., 1998) and possibly transmits CNS.

Country	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Mycoplasma spp*	Environmental *Streptococcus spp*	CNS*	Environmental pathogens	Reference
Iran	-	-	48.75%	-	-	-	Ghazaei, 2006
Mexico			9.92%				Infante., *et al*., 1999

Country	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Mycoplasma spp*	Environmental *Streptococcus spp*	CNS*	Environmental pathogens	Reference
Argentina	2.0%	0.3%	-	-	25.3%	-	Calvinho., *et al*., 2001
Hungria	32.5%	-	-	12.8%	41%	6.8%	Jánosil & Baltay, 2004
Netherlands	-	-	-	-	10.8%		Sampimon *et al*., 2009
Wisconsin	-	-	-	-	12.8%		Pantoja, 2009
Canada	74%	4.4%	SD	-	-	-	Richard., *et al*., 2010
Germany	5.01%			8.7%	17.17%		Schwarz., *et al*., 2010

(*)Coagulase-negative *Staphylococci* (CNS)

Table 1. Pathogen prevalence in Bovine Milk from some productive regions

Country	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Mycoplasma spp*	*Staphilococcus* Coagulase-Negative	Reference
Pennsylvania, USA	150 000 to 700 000 cells/ml				Erskine R.J. *et al*., 1987
Hungary	400 000 cells/ml				Jánosi & Baltay, 2004
Mexico			465 000 cells/ml		Miranda-Morales RE *et al*., 2008
Netherlands				109,000 cells/ml	Sampimon *et al*., 2009
Wisconsin, EEUU	600,000 cells/ml			190,000 to 519,000 cells/ml	Pantoja, 2009
Canada					Richard *et al*., 2010
Germany	>100 000 cells /ml				Schwarz D, *et al*., 2010

Table 2. Somatic cell count (SCC×1000 cells/ml) associated with the mastitis causing microorganism in different countries.

Environmental mastitis pathogens

Streptococcus spp are among the outstanding environmental pathogens as well as *E. coli* and *Corynebacterium* spp. Environmental *Streptococcus* spp are present in dairy herds causing clinical and subclinical mastitis, its presence has been exacerbated due to the increasing implementation of control strategies against contagious pathogens such as *Staphylococcus aureu*s. These programs had reduced the contagious mastitis incidence, however, they had

low effect on the mastitis caused by *Streptococcus* spp, catalase-negative cocci, and by environmental coliform bacteria which affect the udder. Among *Streptococcus* spp, *Streptococcus uberis* (*S. uberis*) is the most frequent as bovine udder pathogen (Olde Riekerink *et al*., 2008). Moreover, the dairy environment is a determinant factor for mastitis development due to *S. uberis* and *Streptococcus dysgalactiae* subsp. *dysgalactiae (S. dysgalactiae*), and stabled dairies are in greater risk than those held in open pastures (NMC, 1999). Other *Streptococcus* spp related in lesser amount to bovine mastitis are *Streptococcus parauberis* (*S. parauberis*), *Streptococcus salivarius* (*S. salivarius*), and *Streptococcus sanguinis* (*S. sanguinis*) (Whitman, 2009). Some Enterococcus such as *Enterococcus faecium* (*E. faecium*), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus saccharolyticus* (*E. saccharolyticus*) (Østerås, *et al*., 2006). *Aerococcus viridans* (*A. viridans*) has been also related to mastitis but its role has not been elucidated yet (Devriese *et al*., 1999; Zadoks *et al*., 2004). In Hungary, Jánosi1 and Baltay, (2004) determined that the environmental caused mastitis by *Streptococcus* sp and *E. coli* had a prevalence of 12.8% and 6.8 % respectively.

The environmental pathogens, by themselves, are not enough frequent and persistent to cause mastitis or as a significant elevation of somatic cells counts (SCC) of bulk milk (values over 400,000 cells/ml). However, 66% of mastitis caused by environmental *Streptococci* and 85% of those caused by coliform bacteria, display clinical presentation. Therefore, losses due to this type of mastitis can reach substantial amounts even in herds with low SCC (<300,000 cells/ml), mainly due to a high incidence of clinical mastitis as it has been estimated around a 46% of clinical mastitis per year in herds with bulk milk SCC counts of less than 200,000 cells/ml

3. Somatic cell counts (SCC)

Throughout the world in the last ten years, udder health programs have been increasing (Godkin *et al*., 1999; Østerås *et al*., 1998; Plym 1996a; Plym et *al*., 1996b; Sargeant *et al*., 1998), and regarded as a critical production issue on dairy farms. In Europe, the European Economic Community (EEC) since 1998 does not recommend consumption of milk with SCC over 400, 000 cells/ml. In North America the limit has been established at 750, 000 (USA) and in Canada at 500, 000 cells (Sargeant *et al*., 1998).

Somatic cells are, in great quantity, cells of the immune system (80% in uninfected quarters, and 99% in quarters with mastitis) (Sordillo *et al*., 1997). They are part of the natural defense mechanisms, including lymphocytes, macrophages, polymorphonuclear and some epithelial cells (Pillai *et al*., 2001). Somatic cells are therefore a reflection of the inflammatory response to an IMI. Somatic cell counts are often used to distinguish between infected and uninfected quarters according to the general agreement between infection status and the inflammatory response to infection reflected as an increased SCC. As with any diagnostic test, errors will occur when solely depending on a single test. To minimize error, diagnostic test parameters such as sensitivity & specificity are calculated at various cut-off values in the continuum SCC (Schepers *et al*., 1997). In North America and Europe the SCC for an uninfected quarter is approximately 70, 000 cells. There is of course variation around this mean; its value can increase with age, decreasing milk production and days in milk period (Schepers *et al*., 1997).

Hence, to be able to distinguish between infected and uninfected quarters a cut-off of approximately 200, 000 to 250, 000 cells is accepted (Dohoo *et al*., 1991; Laevens *et al*., 1997; Leslie *et al*, 1997; Schepers *et al*, 1997). At this cut-off value, diagnostic sensitivity is approximately 75%, and specificity approximately 90% (Schepers *et al*., 1997). The 200, 000 cells cut-off is not considered a physiological cell concentration in milk able to distinguish between healthy and unhealthy udders, but it is a practical value under field conditions (minimizing diagnostic error). Erskine *et al.* 1987, evaluated 32 dairy herds, 16 with low SCC less than or equal to 150, 000 cells/ml and 16 with high SCC greater than or equal to 700, 000 cells/ml. From the 16 herds with low SCC, S. *agalactiae* was isolated in two herds (12.5%), and *S. aureus* was isolated from seven herds (44%). Moreover both microorganisms were found in all of the herds with high SCC, a program of post-milking teat dipping and treatment of all cows at the beginning of the non-lactating period was practiced in the herds with low SCC. Whist *et al*. (2007) reported low SCC in milk from heifers having *Streptococcus dysgalactiae* IMI and in non-infected glands the results indicated that SCC were high (between 50,000 and 100,000 cells/ml) during the immediate postpartum period, within the next 5 days after calving.

4. Bulk tank milk (BTM) SCC

BTM SCC is a general indicator of the udder health in a herd and it is also regarded as an indirect measure of milk quality (Schukken *et al.* 2003). Elevated SCC, are correlated with changes in milk composition, casein and more serum-derived whey proteins, as well as increased proteolytic and lipolytic activities (Auldist & Hubble, 1998). SCC may, however, vary greatly depending on factors such as number of lactations, stage of lactation, season and milking frequency (Harmon, 1994; Pyörälä, 2003). In BTM, where the total volume of milk will dilute effects from affected quarters, SCC appears to be less sensitive and specific as a biomarker for milk quality, e.g. suitability for cheese production (Leitner *et al.,* 2006).

Bulk tank milk SCC assist in directing milk quality control programs and assist with the identification of risk factors in herds. The production of milk with low bacterial counts starts at the farm and is influenced by many procedures related to farm management practices. At the farm level, microbial contamination of BTM occurs through three main sources; bacterial contamination from the external surface of the udder and teats, from the surface of the milking equipment, and from mastitis organisms within the udder (Murphy & Boor, 2000). The levels and types of microorganisms in BTM provide valuable information on the hygienic conditions during the steps of milk production. The microbiological count methods are used to monitor hygienic quality of raw milk including the total aerobic count (TAC). TAC is the most common method for the assessment of bacterial quality of raw milk, it estimates the total number of bacteria present at the farm´s pickup time, providing an overall hygienic milk-quality measure; however, it is limited for the identification of the bacteria contamination source. An alternative has been the standard plate count (SPC) and the preliminary incubation count (PIC), a selective count is measuring psychotropic bacteria, which will grow and multiply under improper refrigeration conditions. These organisms can create undesirable odors and off-flavors. Many psychotropic bacteria can also produce heat-stable enzymes that will survive pasteurization degrading and reducing milk and milk

products during shelf-life (Hayes & Boor, 2001). The laboratory pasteurization count (LPC), another selective count, estimates the number of thermoduric bacteria, mainly from the surfaces of poorly cleaned farm equipment that will survive a laboratory-scale batch pasteurization process. Thermoduric bacteria have been associated with spoilage of pasteurized milk. The Coliform count (CC) measures the number of coliform bacteria in milk, organisms primarily coming from the cow's environment, therefore high CC will give an estimation of the production sanitary status and practices. Coliforms can also incubate on residual films of improperly cleaned milking equipment (Reinemann *et al.,* 2003).

The results from a case–control study indicated that TAC and PIC were mostly related to cow and stall hygiene, whereas LPC and CC were related to equipment hygiene (Elmoslemany *et al*., 2009; Jayarao *et al*., 2004), and included among the bacteria groups associated with bovine IMI are *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp, Streptococcus spp, Escherichia coli,* and SCN.

5. Prevalence of mastitis pathogens and somatic cell counts

In Mexico, the prevalence of mastitis pathogens in BTM SCC from 224 milk samples of 112 herds was as follows; *Mycoplasma* spp were isolated from 62 herds (55%), *S. aureus* from 34 cattle barns, *S. uberis* and CNS were isolates from milk from 42 herds (37.5%) and from 43 (38.3%) bulk tank milk samples. The geometric mean of SCCs was 465, 000 cells/ml. No significant differences were observed in SCCs between *Mycoplasma* spp, *S. aureus* and *Streptococcus* spp positive and negative herds (P>0.5) (Miranda-Morales, *et al.,* 2008).

In Latin America, few data had been carried out regarding microorganism's prevalence and SCC in cases of clinical and subclinical mastitis. Nonetheless, regarding bovine mastitis, Calvinho *et al*, (2005) assessed the primary pathogens prevalence, and its relation with the general udder health status in Argentina from 1983 to 2001. The subclinical mastitis showed a prevalence of 25.3% of *S. aureus* in the 80's and through the years it has been decreasing until a level of 13.9% in 2000. This situation was also observed regarding *S. agalactiae,* which has been reducing its prevalence from 8.8 to 1.6%; *Streptococcus* spp from 19.3 to 6.5% and coliforms from 2.7 to 2%. The prevalence observed for the same pathogens causing clinical mastitis, were low prevalence levels for *S. aureus , S. agalactiae* and coliforms respectively from 34.45 to 29.2%, 13 to 3.9%, and from 20 to 4.4%. In contrast the CNS, *S. dysgalactiae* and *Streptococcus* spp registered rising prevalence from 2.1 to 12.7%, 1.7 to 15.9%, and from 6.4 to 19.8% respectively. This situation was also seen among SCC registering levels of 400, 000 to 900, 000 cells/ml in the 80's, since after a sustained decrease in the SCC from BTM in recent years; in 2004 ranging around 300, 000 cells/ml, and in 2005, an average of 384, 000 cells/ml (SAGPyA, 2005). The producers have been implemented systematically control programs based on hygiene and antibiotic therapy, there has been a decrease in the prevalence of contagious pathogens and environmental relative increase, however it should be noted that the SCC values remain high compared with those of countries with high dairy development. In Perú, Ortiz, *et al*., 2006, assessed the SCC in dairy herds of different technological levels in Arequipa, milk samples were collected twice in 2005. The stables were stratified according to

their technological level in high, medium and low. The general average of SCC were 505 x 103 x 103 ± 150 cells/ml, and significant differences between technology levels were identified as SCC were 353, 559 and 603 x 103 cells/ml for high, medium and low, respectively ($p < 0.05$), feature explained by the dilution of somatic cells in a greater volume of milk and a more rational application of best practices to prevent and control mastitis in the most sophisticated stables. On the other hand, limited access to training adversely affects low-technology. In a study by Moraga *et al* (1994) in Chile, the prevalence of bovine mastitis in the years 1972 to 1992; subclinical mastitis in 1972 was 45.42%, and by 1992 the prevalence had reduced to 38.65%, traduced on a 14.90%. Regarding clinical mastitis a continuous prevalence reduction of 12.86% from 74.41% to 64.84% was determined during the same period. Furthermore, the SCC were reduced from 1,983,310 cells/ml to 1,055,240 cells/ml, in these 20 years. These decrements on the severity of subclinical mastitis obeys the current control measures spread in the early 70's, such as post-milking disinfection of teats and drying therapy used in the 66.7% of the farms studied as well as the general infrastructure improvement. Finally despite the progress, acceptable control mastitis levels have not yet been reached.

In Mexico, Infante, *et al*., (1999), observed in a commercial dairy herd (282 cows) in lactation a sudden atypical clinical mastitis outbreak with 28 cases of severe purulent mastitis, hard swollen mammary glands and lacking systemic signs of illness. The treatment non- responsive cases (Table 1) suggested the spreading through the milking machine and other management practices, further cultures determined the presence of *Mycoplasma californicum* and *Mycoplasma canadense*. A second study performed by Miranda-Morales, *et al*., (2008), revealed that *Mycoplasma* spp were present in the 55% of the 62 herds included, also that *S. aureus* was present in the 30% of cattle barns and that *S. uberis* and CNS were present in 42 herds (37.5%) and 43 herds (38.3%) according to the BTM samples, respectively. The geometric mean of SCCs was 465, 000 cells/ml and no significant differences were observed among *Mycoplasma* spp, *S. aureus* and *Streptococcus* spp positive and negative herds ($P > 0.5$) (Table 2 and 5).

Overall, prevalence of mastitis is over 10%, in samples of direct milk *Staphylococcus aureus* has a prevalence >30% in contrast to an <5% prevalence of *Streptococcus agalactiae*, and a prevalence between 15 and 41% has been reported for CNS. *Mycoplasma* has been reported in a few prevalence studies and environmental mastitis pathogens have an average prevalence of >15%. However in BTM *Staphylococcus aureus* have registered consistently high figures from 30% and up to 74%, followed by the prevalence values of *Streptococcus agalactiae* around 40%, and in BTM *Mycoplasma* spp had variable prevalence figures ranging from 50% to 85%. Regarding SCC, values of 100, 000 – 700, 000 cells/ml are associated to the presence of *Staphylococcus aureus* and *Streptococcus agalactiae.* For *Mycoplasma* spp, SCC values are > 200, 000 cells/ml, and SCC of 100, 000 and up to 500, 000 cells/ml are associated to CNS infection. Currently, in America, BTM-SCC values are around > 200, 000 cells/ml, therefore milk quality requirements are barely meet except for some regions that had achieved SCC levels of < 200, 000 cells/ml, and low prevalence of mastitis associated pathogens. Therefore, herd overall studies are mandatory for mastitis control programs including duration of lactation, season, milk production and parity. But will also be guided by the prevalence of mastitis pathogens and by the, geographic region and production practice.

Country	BTM	SCC	Reference
Seattle, USA	93	533 000 cells/ml	Fox L.K. *et al.*, 2003
Argentina	7358	384 000 cells/ml	SAGPyA, 2005
Peru	15	500 000 cells/ml	Ortiz Z.C. *et al.*, 2006
Argentina	51	250 000 cells/ml	Vissio, C., *et al.*, 2007
Mexico	112	465 000 cells/ml	Miranda-Morales R.E., *et al.*, 2008

Table 3. SCC values of BTM milk samples associated with mastitis pathogens of some regions worldwide.

Reference	No. of Dairy herds	No. of bovine	Gland infected (%)
Zurita., *et al*., 1972		1 137	48,81%
Moragay., *et al*., 1993	30	2 321	41,10%
Chaves., *et al*., 1996		19	37%
Calvinho., *et al*., 2001		86	62,8%
Sampimon., *et al*., 2009	49	1 960	10,8%
Castillo., *et al*., 2009		2 116	72,61%

Table 4. General overview of mastitis prevalence.

Reference	Dairy herds studied	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Mycoplasma* spp
Kunkel, 1985	2346	-	-	*1.3%*
Guillemette., *et al*., 1992		-	*6%*	-
Schoonderwoerd., *et al*., 1993		-	*43%*	-
Keefe., *et al*., 1997		*70%*	*18%*	
Kirk., *et al*., 1997	267	-	-	*78.2%*
Fox., *et al*., 2003	664	-	-	*14%*
Sato, 2004	118	*71.6%*	-	-
Sato, 2004	40	*27.55%*	-	-
Riekerink., *et al*., 2006	258	74 %	1.6 %	1.9%
Howard, 2006	7	57.1%	-	-
Ghazaei, 2006	48	-	-	85,25%
Miranda-Morales., *et al*., 2008	112	30%	-	55%
Richard & Riekerink., *et al*., 2010	226	74%	4%	-

Table 5. Prevalence of contagious mastitis pathogens in BTM.

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