More Value From Milk: Value-add Opportunities

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Introduction

Milk provides a complete diet for the neonate, which initially is unable to collect, chew and digest solid food. In addition, it conveys immune factors to the newborn and depending on the species (i.e., if *in utero* transfer of immune factors occurs or not) it may be the only source of immune factors at the start of the neonates life; such as is the case with bovine calves. Colostrum, the very first milk produced around parturition, is particularly rich in immune factors and nutrients. Therefore, milk is essential for the survival of the mammalian newborn (Stelwagen et al., 2009).

Lactation is, thus, a critical, but often overlooked, part of the reproductive process in mammals. Indeed, evolutionary, milk production may have originally been part of a reproductive strategy of egg-laying premammalian animals (Capuco and Akers, 2009; Oftedal, 2012). Briefly, about 310 million years ago (mya) Synapsida had evolved into animals that laid soft-shell, non-calcified eggs, whereas the other branch splitting of Amniota, the Sauropsida, evolved into hard-shell egg laying animals, giving rise to birds, turtles and crocodiles. The Synapsida eventually, about 160 mya, evolved into the current-day mammalian species of Eutheria (placental), Metatheria (marsupials) and Monotremata (egg laying).

Early mammals were still egg-laying, and Monotremes continue to do so even now. Because soft-shell eggs would be extremely sensitive to drying out, it is believed that the early "milk" secretions were produced by skin glands to keep the soft-shell eggs from drying out (Oftedal, 2012). Therefore, the mammary gland is likely to have evolved from skin glands. Skin glands produced secretions abundant in protective factors, including antimicrobials and overtime these secretions became more nutrient-dense and started to provide a source of nutrients and immune factors for the offspring. Even to date, milk, and in particular colostrum, remain not only rich sources of nutrients, but also of immune factors (Smolenski et al., 2007, 2014; Wheeler et al, 2012).

In the modern dairy cow milk production has increased well beyond that necessary to feed its calf, as a results of continuing improvements in genetic selection, feeding and nutrition and management. Allowing milk to become an economic commodity, to provide a range of dairy products for human consumption. However, increasingly milk is not only recognized for its nutritional properties, but also as a source of a wide range of potentially high-value ingredients that have specific, but diverse, bioactive properties (Korhonen and Pihlanto, 2003; Wakabayashi et al., 2006; Stelwagen, 2011). In this paper a number of these bioactives and possible opportunities will be discussed, including colostrum, milk protein, milk fat, milk lactose, and milk minor components.

Milk Composition and Bioactives

At a basic level milk consists of five components: fat, protein, carbohydrates, minerals and water. However, the relative proportions of these components can vary considerable among different species (Larson, 1985). Water is usually by far the largest fraction, in cows approximately 88%. The notable exception to this is the extreme energy-dense milk of certain marine mammals, where fat is the most abundant component (often >50%).

The fact that the gross milk composition only consist of five major components, hides the fact that in reality, milk, just as blood, is an enormously complex biological fluid made up of thousands of specific components such as fatty acids, a vast array of proteins and peptides, including various whey proteins, caseins, many different enzymes and immune-related proteins and peptides, lactose, oligosaccharides, minerals, vitamins, phospholipids, nucleotides, etc. Moreover, the composition of milk may change considerable during the course of lactation (Stelwagen et al., 2009; Ballard and Morrow, 2013) and can also be significantly influenced by the diet of the mother (Sutton, 1989) and disease status of the gland (Auldist and Hubble, 1998).

The complexity of the milk composition and the fact that its evolved biological role is to provide nutrients and immune factors for growth, development and health, makes it a logical target for "mining" of milk bioactive substances. A bioactive is defined here as any biological molecule or fraction that elicits a biological response. Milk may contain hundreds to thousands of such bioactives (Ballard and Morrow, 2013) that can play a role in biological process ranging from antimicrobial and antiviral activity, to bone health, hypertension and anticarcinogenic activity and may find commercial applications as nutraceuticals, cosmetics, and special high-value food ingredients for infant nutrition and other targeted nutritional formulas (Wakabayashi et al., 2006; Korhonen, 2009; Stelwagen et al., 2009; Hill and Newburg, 2015).

Colostrum

Colostrum is the very first milk produced at the onset of lactation and is rich in protein and immune and immune-facilitating factors, providing nutrients and immune protection to the neonate. It should come as no surprise, therefore, that colostrum is a rich source of bioactive factors, ranging from immunoglobulins molecules, growth factors, lactoferrin and enzymes to yet to be identified factors (Uruakpa et al, 2002; Stelwagen et al., 2009).

Because high-quality colostrum is only produced during the first two days of lactation in cows the total quantities available are insufficient to commercially extract individual components from colostrum. Instead, whole colostrum (and sometimes de-fatted colostrum) is commonly processed into high-value colostrum powder and sold to consumers as powders, tablets or capsules. Because, it is easy to fraudulently dilute colostrum with milk, to increase the quantity of "colostrum", it is sold based on its immunoglobulin G (IgG) content; the higher the IgG content, the higher the colostrum quality. Commercial products commonly have an IgG content between 15 and 20%. Colostrum is one of the best recognized examples of a dairy value-add product, with a price in the order of US$ 50 to US$100 per kg (2.2 lbs) powder.
Colostrum is sold for it functional properties, targeting general immune and infection protection, gut health and growth and tissue repair (Uruakpa et al. 2002). In particular, its role in gut health has received considerable research interest. This is perhaps not surprising, since the gut plays an important role in colostrum absorption in the newborn. In the young calf, the tight junctions

between the gut epithelial cells are only permeable during the first 24 to 48 h, allowing uptake of the intact large immunoglobulin molecules (i.e., antibodies). Of course this initial gut permeability, also puts the newborn at risk of pathogens entering from the gut and gut closure occurs rapidly during the first 48 h of life. It appears that colostrum contains, yet to be identified, factors that facilitate gut closure and tight junction formation (Playford et al., 1999; Prosser et al., 2004). Colostrum may help to prevent and treat "leaky gut" syndrome, as a result of heat stress, use of nonsteroidal anti-inflammatory drugs, or high-performance sports (Playford et al., 1999, 2001; Prosser et al., 2004).

Due to the high value of colostrum, it may be tempting for farmers to sell as much as possible. However, it is pertinent that at all times the first priority is to supply newborn calves with an adequate amount of high-quality colostrum. Otherwise animal welfare concerns will unnecessarily tarnish the colostrum market. A market that is predicted to have a compound annual growth rate (CAGR) of 3.2% over the next 10 years and reach a global market value of almost US$ 2 billion (bn) by 2026, with infant food and probiotics being the key growth markets (FMI, 2016).

Milk Protein

Protein in milk consists of two major classes, caseins and whey proteins. It is the caseins that are required for cheese production. For this reason, traditionally caseins have been considered the most valuable milk proteins and whey protein together with the whey fraction of the milk was considered a "waste product", and was, and in some places still is, either returned to the farm for use as calf or pig feed, or it was sprayed on paddocks near the dairy factory as a "fertilizer". Nowadays, whey proteins are a highly valued protein source for human nutrition, and may arguably hold more value then casein.

WPC, WPI, WPH

The milk whey fraction, not only contains whey protein, it also contains the lactose, some residual lipid, mineral fractions, and most of the "minor" components and bioactives. The whey fraction of milk is processed into whey powder and marketed as whey protein concentrates (WPC), whey protein isolates (WPI) or as whey protein hydrolysates (WPH). In WPC all the whey components are present and the abbreviation WPC is usually followed by a number, often 80, indicating the level of protein in the powder (e.g., WPC-80 has 80% whey protein). In WPI the whey is further processed to remove most of the lactose and minerals through ultrafiltrattion, resulting in a higher protein content (>90%) in WPI, and therefore a more high-value product. Both WPC and WPI can be processed into hydrolysates, in which the intact whey proteins have been partially broken down into peptides, using heat, acid or enzymes. Whilst hydrolysates often have a bitter taste, this adverse attribute is offset by improved digestion and absorption following consumption. For this reason WPH are often used in specialized medical formulas.

Whey proteins powders, be they WPC, WPI or WPH, are now highly valued for their nutritional properties and have given rise to an ever-increasing body of research. For example, a recently published pilot study suggested that whey protein supplementation may have a role to play in preventing or managing Parkinson's disease (Tosukhowong et al., 2016). Patients with Parkinson's disease usually exhibit an elevated plasma level of homocsytein and reduced level of glutathione, a powerful antioxidant. Dietary supplementation with whey protein increased the

level of glutathione in plasma and decreased that of homocysteine. Further research is necessary to show if this will ultimately lead to improved clinical outcomes, but it highlights the potential of whey protein. There are numerous scientific studies in the literature reporting on various aspects of dietary whey proteins. These are too diverse and numerous to discuss in any detail in this paper and, moreover, associated commercial applications may still take a long time to materialize. Therefore, in this paper only two major current commercial applications of whey proteins will be discussed.

Infant and Other Specialized Nutritional Formulas

It must be emphasized that breast milk is the most complete and desirable food for newborn infants. Unfortunately, for biological or economic reasons, breast milk is not always an option, and a high-quality infant formula then provides the best alternative nutritional strategy. All high-quality infant formulas aimed at normal, healthy infants, contain bovine whey protein as the sole protein source. In contrast to bovine milk, in human milk whey protein constitutes the largest milk protein fraction (whey:milk = 80:20 in early lactation human milk). Therefore, a bovine whey-dominant infant formula comes closest to the human milk protein profile. Also, for an infant, whey protein is much easier to digest than casein protein.

In recent years, the market for infant and baby food in China and other Asian countries has seen very strong growth. The CAGR for this market segment in Asia has been estimated at approx. 8% between 2014-2019 (Coriolis, 2015). Increasingly, as people live longer and the average age of population increases in many countries, there is a growing market for nutritional supplement formulas for elderly, again WPC is one of the key ingredients of such formulas. Particularly since whey protein may help to prevent age related loss of muscle mass and strength, due to its unique amino acid composition (see next section) (Bjorkman et al., 2011; Devries and Phillips, 2015)

Sports and Exercise

The biggest market for why protein, by far, is the sports nutrition supplement market. Worldwide health and sport nutrition retail outlets sells “muscle boosting” powders in various disguises. Regardless of the brand, nearly all are based on milk whey powder and sometimes also colostrum powder. This is no coincidence, as there is a vast body of scientific research showing that whey protein has a superior amino acid composition that promotes maintaining and gaining muscle mass and strength (Wilkinson et al., 2007; Bjorkman et al., 2011; Volek et al., 2013; Devries and Phillips, 2015; Phillips, 2016). This is largely due to the high content of the amino acid leucine (Volek et al., 2013; Phillips, 2016), which is not only a key amino acid required for muscle synthesis, it also is the trigger of muscle synthesis (via activation of the rapamycin signalling cascade) (Phillips, 2016).

Not only may whey milk powder help with muscle maintenance and synthesis, it may also help to address another adverse condition frequently observed with high-level sports and exercise, dehydration. A recent study, comparing 12 different commercially available drinks with still water, in terms of their capacity to rehydrate the human body, indicated that full-fat milk and skim milk were the best at rehydrating the body (Maughan et al., 2016). In fact, both were as good as a commercially available pharmaceutical rehydration fluid. Very similar results were obtained in another recent study, indicating that skim milk was superior over water and a

commercial carbohydrate-electrolyte drink (Powerade™), in rehydrating subjects after exercise (Seery and Jakeman, 2016). The exact mode of action of the milk protein is not yet fully understood. The protein needs to be digested and this may help to increase the retention time of the liquid, or factors in milk that help prevent gut permeability (Prosser et al., 2004), a key factor in dehydration, may play a role.

The market for sport nutrition supplements continues to grow at a rapid rate worldwide. Data recently released by Euromonitor International (2016), revealed that in the USA alone the market for sports protein powder is expected to increase from US$ 4.7 bn in 2015 to US$ 7.5 bn in 2020, that for sports protein bars from US$ 0.5 bn to US$ 0.6 bn, and sports protein ready-to-drink (RTD) from US$ 0.8 bn to US 1.1 bn. Combined more than US$ 9 bn in 2020, up 35% on 2015. Of course most of the protein used in these markets will be whey protein. Given the scientific data underpinning whey protein's role in muscle gain and maintenance it is no surprise that whey powders are very lucrative value-add products.

Milk Fat

Milk fat consists mostly (approx. 98%) out of triglycerides, molecules that have a glycerol with three fatty acid side chains (Jensen, 2002). Most of these fatty acids are saturated and because of this, milk fat is generally perceived to be unhealthy and bad for cardiovascular health, although increasingly it is becoming apparent that such fears may be unfounded (Huth and Park, 2012). Nevertheless, the triglyceride milk fraction itself offers little scope to add value to milk, other than its use in butter and regular full-cream dairy products.

Conjugated Linoleic Acid (CLA)

However, milk does contain relatively large amount of CLA and, in particular, the *cis-9, trans-11* CLA isomer, due to rumen metabolism of linoleic (converted to CLA) and linolenic acid (converted to CLA-precursor vaccenic acid). Because, linoleic and linolenic acid are reasonable abundant in fresh green forages, CLA levels in milk are highest in milk from cows on pasture (Auldist et al., 2002). Conjugated linoleic acid is a poly-unsaturated fatty acid (PUFFA) and, as such, considered one of the "healthy" fatty acids. Over the years, CLA in particular, has received considerable attention for its health promoting properties. A considerable and wide-ranging body of *in vitro* and *in vivo* research in both animals and humans has shown beneficial effects in relation to, certain cancers, diabetes, atherogenesis, general immune modulation, and especially obesity, with CLA promoting lean growth (Parodi, 1999; Benjamin and Spener, 2009).
The well-documented effect of CLA on lean growth not only offers opportunities to manage obesity, but also has made CLA popular among body builders and weight lifters. These latter groups mostly use chemically synthesized CLA, which is freely commercially available. However, with an increasing trend of consumer preferences for more "natural" (The Nielson Company, 2015), CLA-rich products derived from milk may offer a promising opportunity.

Despite the overwhelmingly positive research support for the beneficial properties of CLA, one criticism is that most of these studies have been with such high dose levels of CLA, that it would be impractical to derive such benefits from CLA in food. However, most of the trials to date have been of short duration and a few long-term (>1 year) trials with lower dose levels show promise, but more research is required (Benjamin et al., 2015).

Docosahexaenoic Acid (DHA)
Docosahexaenoic acid is an omega-3 fatty acid and another PUFA that has an ever increasing body of scientific research underpinning its beneficial properties in health and many developmental processes, including brain development in the growing fetus (Koletzko et al., 2008). Moreover, recently the first randomized double-blind clinical study on the effects omega-3 (incl. DHA) on maintaining and improving brain function in elderly was published (Witte et al., 2014).

Unfortunately, because many dietary fatty acids undergo biohydrogenation in the rumen, ruminant milk contains very little to no DHA. Feeding cows fish oil, may increase DHA levels in the milk (Vahmani et al., 2013) , however, because of dwindling fish and krill stocks this is not a sustainable practice long-term. In addition, milk from cows fed fish-oil may have a "fishy" odor. Instead, algae that can be grown very rapidly under controlled conditions and spray-dried into a powder may provide a much more sustainable way to increase DHA levels in milk. Recently, results from a field trial on a commercial dairy farm, under pasture grazing conditions, in which part of the herd received an DHA-rich algal supplement (ALG-Rich™, Altech), showed that cows can produce milk with high DHA levels (Figure 1A). With the dose level used in this trial (112.5 g/d/cow) the level of DHA in milk increased from undetectable levels to 8.7 mg/100g milk after 6 weeks of supplementation, sufficient to provide approx. 20% of a child's recommended daily intake (RDI) in a single serving of standard whole milk. However, higher dose levels will result in a higher level of DHA in milk.

Interestingly, the CLA level in the milk, already high in both groups because the cows were on a pasture diet, was increased by a further 44% in the algae-supplemented group (Figure 1B). Moreover, vaccenic acid, a precursor of CLA was also higher (47%) in the supplemented group (data not shown).

Although marine-extracted DHA is increasingly added to foods, including milk, during processing, letting the cow herself incorporate DHA into the milk, is more natural, as it will be mostly in the triglyceride form; this may also improve bioavailability, although the latter requires more research.

Milk Carbohydrates
Milk sugars or carbohydrates perform an important function in the synthesis of milk. They act as the main osmoregulatory molecules in the secretory vesicles within mammary secretory epithelial cells and, as such, they draw in water from the surrounding cytoplasm within the cell. Subsequently, the vesicles fuse with the apical membrane of the cell to release their content into the alveolar space, adding the water component to the milk; thus, milk sugars are the main determinants of milk volume.

Oligosaccharides are large complex carbohydrate molecules that are particularly high in human milk. They play an important role in host-defense by binding to the surface of pathogenic bacteria in the gut, thereby preventing them and their toxins from binding to receptors in the gut wall. They also can act as prebiotics and, as such, act as a selective growth substrate for beneficial gut bacteria (Newburg, 1996).

Unfortunately, whilst prominent in human milk, oligosaccharides are much less abundant in bovine milk, therefore limiting their commercial application. In bovine milk the disaccharide lactose, consisting of two linked glucose molecules, is by far the most prominent carbohydrate in bovine milk. Milk lactose is a valuable, sought after component extracted from bovine milk for application as:

- Pharmaceutical ingredient. Purified lactose is commonly used as a filler or diluent in pharmaceutical pills or tablets and it is also used to make the synthetic inert sugar lactulose, a common treatment for constipation (Booij, 1985);
- Infant nutrition. Lactose is the main ingredient in infant formula, with most formulas ranging from 50 to 55%;
- Dietary fibre or prebiotic ingredient. Lactose is used for the manufacture of galacto-oligosaccharide (GOS), used increasingly as a source of dietary fibre and as a prebiotic in infant and other formulas (Ghisolfi, 2003; Champ and Hoebler, 2009).

Minor Milk Components

Milk contains thousands of minor components, such as vitamins, minerals, small proteins and peptides, enzymes, phospholipids, and many more. Although most of these have bioactive properties (Ballard and Morrow, 2013), this does not automatically mean they can be simply extracted from milk as potential value-added products. Many of these molecules have intrinsic and support roles and may serve, for example, as enzymes or signalling molecules facilitating specific biological processes in the mammary gland or in the neonate. Even molecules that have direct commercially attractive bioactive properties, may simply be not present in the milk in sufficient quantities to warrant commercialization. Further, whilst lab-scale extraction may be possible, often scale-up to commercial level may be prohibitive, due to cost or health and safety reasons (e.g. if large quantities of solvents are required). Finally, if a potentially commercially attractive bioactive factors is identified, often the first action is to isolate the molecule from milk, with the intent to "stick it in a bottle and sell it", assuming a market readily exists. However, as indicated earlier, milk is a very complex fluid, and often when the bioactive is removed from its milk matrix, it shows no or greatly reduced bioactivity. This is why it is often better to extract a milk fraction, enriched with the bioactive of interest, rather than trying to fully purify the bioactive itself from the milk.

These difficulties probably explain why to date so few bioactives from milk have been actually commercialized. Bovine milk lactoferrin is the shining example of an actual commercially profitable value-add components derived from milk (Wakabayashi et al., 2006). However, notwithstanding these difficulties the number of patents filed for a range of milk bioactives is steadily increasing (Figure 2) and provides a glimpse of what might be on the horizon, in terms of value-add opportunities.

Bovine Lactoferrin

Lactoferrin is a small milk whey protein. In human milk it is the second most abundant milk protein (approx. 1.7g/L [0.17%], Vegarud et al., 2000), but in bovine milk it is normally present at very low levels (0.1 g/L [0.01%], Vegarud et al., 2000), except in colostrum, very late-lactation milk and in mastic milk (Stelwagen et al., 2009). The fact that it is elevated in bovine milk at these times, readily suggests that it may have an immune protective function. Indeed, lactoferrin, an iron binding glycoprotein, plays a multifaceted role in host defence and has

antimicrobial, antiviral, anti-inflammatory, antioxidant, and general immune stimulatory activities. The scientific evidence underpinning the various aspects of the biological activity of lactoferrin is substantial (El-Loly et al., 2011; Garcia-Montoya et al., 2012); a simple Pubmed search for "lactoferrin" and "milk" yielded 1571 scientific publication, as of 16 November 2016.

Commercial applications of lactoferrin are in human, pet and fish food, cosmetics (skin care) and oral care (tooth paste, mouth wash, chewing gum) (Wakabayashi et al., 2006). Given that lactoferrin is the second most abundant protein in human milk, it is not surprising that infant formula is now a rapidly growing market for lactoferrin.

Lactoferrin is another low volume, high value milk bioactive, at between US$500 to US$1,000 per kg (2.2 lbs); this compares favourably with whole milk powder (WMP) commodity at approx. US$3.40 per kg (2.2 lbs). Because of the low level of lactoferrin in normal bovine milk, it takes about 10,000 L (22,000 lbs) to produce 1 kg (2.2 lbs) of lactoferrin. Despite this low yield, returns warrant the significant investment in lactoferrin extraction plants, requiring large ion-exchange columns. Several dairy companies in Europe, Japan and the USA and nearly all dairy companies in New Zealand have lactoferrin extraction plants.

Bovine Osteopontin

Osteopontin is another glycoprotein, albeit heavily phosphorylated, that is present in most biological fluids within the body, but the highest level is found in milk. It is a multifunctional bioactive, involved in a range of biological process, from immune modulation to bone formation (Christensen and Sorensen, 2016). Although it is expressed in many tissues throughout the body, it is highly expressed in bone tissue, and this is reflected in its name.

Human milk, compared to bovine milk, has a relatively high level of osteopontin (human milk, 138 mg/L [0.014%]; bovine milk: 18 mg/L [0.002%]; Schack et al., 2009), making infant nutrition an obvious potential market for this bioactive. Similarly to lactoferrin, osteopontin is commercially extracted from milk by large-scale ion-exchange processing. Danish dairy company Aral is marketing an osteopontin product (Lacprodan® OPN-10) as an ingredient for infant formula, to support immune function.

Conclusions

Evolutionary milk has evolved from a skin secretion to protect soft-shelled eggs from dehydration, into a highly nutrient- and immune factor-dense milk that is not only essential to the survival of the neonate, but also contains thousands of bioactive molecules. With ongoing scientific research to elucidate the function and roles of these bioactive factors, as well as ways to extract them from milk in a commercially viable manner, there is great potential to add value to milk in addition to that of the standard dairy consumption products. Such value-add products will be low to medium volume, high-value products. Current examples that are commercially produced and marketed include colostrum, whey proteins lactoferrin and osteopontin, and WPC, WPI and WPH.

References

Auldist, M.J., and I.B. Hubble. 1998. Effects of mastitis on raw milk and dairy products. Austr. J. Dairy Technol. 53:28-36.

Auldist, M.J., J.K. Kay, N.A. Thomson, A.R. Napper, and E.S. Kolver. 2002. Concentrations of conjugated linoleic acid in milk from cows grazing pasture or fed a total mixed ration for an entire lactation. Proc. NZ Soc. Anim. Prod. 62:240-241.

Ballard, O., and A.L. Morrow. 2013. Human milk composition: nutrients and bioactive factors. Pediatr. Clin. North Am. 60:49-74.

Benjamin, S., P. Prakasan, S. Sreedharan, A. D. Wright, and F. Spener. 2015. Pros and cons of CLA consumption: an insight from clinical evidences. Nutr. Metab. (Lond.). 12:4 (20 pages).

Benjamin, S., and F. Spener. 2009. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr. Metab. (Lond.). 6:36 (13 pages).
Bjorkman, M. P., T. K. Pilvi, R. A. Kekkonen, R. Korpela, and R. S. Tilvis. 2011. Similar effects of leucine rich and regular dairy products on muscle mass and functions of older polymyalgia rheumatica patients: a randomized crossover trial. J. Nutr. Health Aging. 15:462-467.

Capuco, A.V., and R.M. Akers. 2009. The origin and evolution of lactation. J. Biol. 8:37 (4 pages).

Champ, M., and C. Hoebler. 2009. Functional food for pregnant, lactating woman and in perinatal nutrition: a role for dieatry fibre? Curr. Opin. Clin. Nutr. Metab. Care. 12:565-574.

Christensen, B., and E.S. Sorensen. 2016. Structure, function and nutritional potential of milk osteopontin. Int. Dairy J. 57:1-6.

Coriolis. 2015. Opportunities for New Zealand dairy products in South East Asia. Part of the New Zealand Government's Food & Beverage Information Project. www.foodandbeverage.govt.nz

El-Loly, M.M., and M.B. Mahfous. 2011. Lactoferrin in relation to biological functions and applications: a review. Int. J. Dairy Sci. 6:79-111.

FMI. 2016. Future Market Insights. www.futuremarketinsights.com/press-release/colostrum-market.

Garcia-Montoya, I.A., T.S. Cendon, S. Arevalo-Gallegos, and Q. Rascon-Cruz. 2012. Lactoferrin a multiple bioactive protein: an overview. Biochim. Biophys. Acta. 1820:226-236.

Ghisolfi, J. 2003. Dietary Fibre and prebiotics in infant formulas. Proc. Nutr. Soc. 62:183-185.

Hill, D.R., and D.S. Newburg. 2015. Clinical applications of bioactive milk components. Nutr. Rev. 73:463-476.

Huth, P.J., and K.M. Park. 2012. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. Adv. Nutr. 3:266-285.

Jensen, R.G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Koletzko, B., E. Lien, C. Agostoni, H. Böhles, C. Campoy, I. Cetin, T. Decsi, J.W. Dudenhausen, C. Dupont, S. Forsyth, I. Hoesli, W. Holzgreve, A. Lapillonne, G. Putet, N.J. Secher, M.S. Symonds, H.P. Willatts, and R. Uauy. 2008. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy; review of current knowledge and consensus recommendations. J. Perinat. Med. 36:5-14.

Korhonen, H., and A. Pihlanto. 2003. Bioactive peptides: new challenges and opportunities for the dairy industry. Austr. J. Dairy Technol. 58:129-134.

Korhonen, H.J. 2009. Milk-derived bioactive peptides: from science to apllications. J. Funct. Foods. 1:177-187.

Larson, B.L. 1985. Lactation. The Iowa State University Press, Ames, Iowa, U.S.A.

Maughan, R.J., P. Watson, P.A. Cordery, N.P. Walsh, S.J. Oliver, A. Dolci, N. Rodriguez-Sanchez, and S.D. Galloway. 2016. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. Am. J. Clin. Nutr. 103:717-723.

Newburg, D.S. 1996. Oligosaccharides and glycoconjugates in human milk: their role in host defense. J. Mam. Gland Biol. Neoplasia. 1:271-283.

Oftedal, O.T. 2012. The evolution of milk secretion and its ancient origins. Animal 6:355-368.

Parodi, P.W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. J. Dairy Sci. 82:1339-1349.

Playford, R.J., D.N. Floyd, C.E. Macdonald, D.P. Calnan, R.O. Adenekan, W. Johnson, R.A. Goodlad, and T. Marchbank. 1999. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. Gut. 44:653-658.

Playford, R.J., C.E. MacDonald, D.P. Calnan, D.N. Floyd, T. Podas, W. Johnson, A.C. Wicks, O. Bashir, and T. Marchbank. 2001. Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. Clin. Sci. (Lond). 100:627-633.

Schack, L., A. Lange, J. Kelsen, J. Agnholt, B. Christensen, T.E. Petersen, and E.S. Sorensen. 2009. Considerable variation in the concentration of osteopontin in human milk, bovine milk, and infant formulas. J. Dairy Sci. 92:5378-5385.

Seery, S., and P. Jakeman. 2016. A metered intake of milk following exercise and thermal dehydration restores whole-body net fluid balance better than a carbohydrate-electrolyte solution or water in healthy young men. Br. J. Nutr. 6:1013-1023.

Smolenski, G.A., M.K. Broadhurst, K. Stelwagen, B.J. Haigh, and T.T. Wheeler. 2014. Host defence related responses in bovine milk during an experimentally induced Streptococcus uberis infection. Proteome Sci. 12:19.

Smolenski, G., S. Haines, F.Y. Kwan, J. Bond, V. Farr, S.R. Davis, K. Stelwagen, and T.T. Wheeler. 2007. Characterisation of host defence proteins in milk using a proteomics approach. J. Proteome Res. 6:207-215.

Stelwagen, K. 2011. Milk biosynthesis and secretion: Protein. In: Fuquay, J.W, P.F. Fox, and P.L.H. McSweeney (eds.). Encyclopedia of Dairy Sciences, Second Edition, vol. 3, pp. 359-366. San Diego: Academic Press.

Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. Wheeler. 2009. Immune components of bovine milk and colostrum. J. Anim. Sci. 87 (Suppl. 1):3-9.

Sutton, J.D., and S. V. Morant. 1989. A review of the potential of nutrition to modify milk fat and protein. Livest. Prod. Sci. 23:219-237.

The Nielson Company. 2015. We are what we eat – healthy eating trends around the world. Global Health and Wellness Report. Pages 1-24.

Uruakpa, F.O., M.A.H. Ismond, and E.N.T. Akobundu. 2002. Colostrum and its benefits: A review. Nutr. Res. 22:755-767.

Vahmani, P., A.H. Fredeen, and K.E. Glover. 2013. Effect of supplementation with fish oil or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. J. Dairy Sci. 96:6660-6670.

Vegarud, G.E., T. Langsrud, and C. Svenning. 2000. Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. Br. J. Nutr. 84 Suppl 1:S91-S98.

Wakabayashi, H., K. Yamauchi, and M. Takase. 2006. Lactoferrin research, technology and applications. Int. Dairy J. 16:1241-1251.

Wheeler, T.T., G.A. Smolenski, D.P. Harris, S.K. Gupta, B.J. Haigh, M.K. Broadhurst, A J. Molenaar, and K. Stelwagen. 2012. Host-defence-related proteins in cows' milk. Animal. 6:415-422.

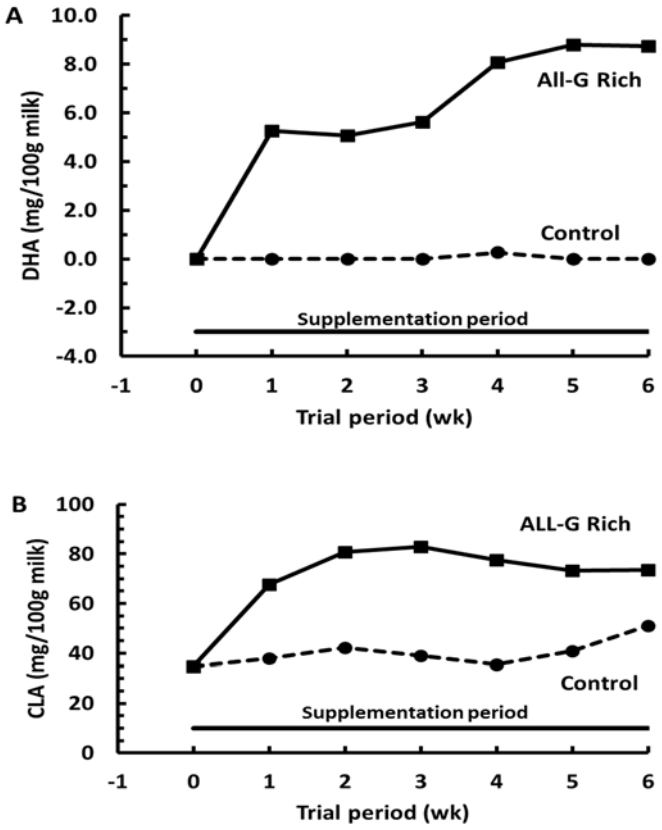

Figure 1. Effect of feeding an algae supplement on DHA (A) and CLA (B) levels in the milk. Cows were part of a commercial herd, managed under pasture grazing conditions in New Zealand. The herd was split into two groups, a supplemented (ALG-Rich™, Alltech; 112.5 g/d/cow, n=139 cows) and a control group (no supplement; n=212 cows). Cows were 184 days into milk at the start of the trial.

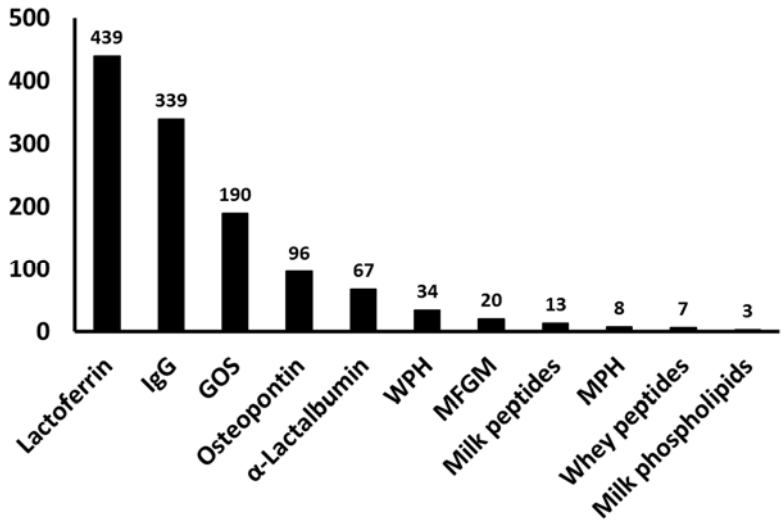

Figure 2. Recent patent activity of milk derived bioactive components, between 2011 and 2015. IgG = immunoglobulin G; GOS = galacto-oligosaccharide (NB: is manufactured from milk lactose); WPH = whey protein hydrolysate (NB: is manufactured from milk whey protein); MFGM = Milk fat globule membrane; MPH = milk protein hydrolysates (NB: is manufactured from milk protein). (Source: DAIRYreporter.com, European Patent Office – Global Patent Index).

Evolution of Mastitis Extension in New Zealand – from SAMM Plan to SmartSAMM

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Summary
In 1993, a national mastitis extension program was developed in NZ to help farmers cope with the new financial penalties for high SCC milk. Known as the SAMM plan, this helped dairy companies achieve substantial improvements in milk quality, with bulk milk SCC for the largest dairy company at the time (New Zealand Dairy Group) dropping by over 100,000 cells/ml between 1992 and 1996. But by 2008, the bulk milk SCC was trending back up towards 250,000 cells/ml. This led to a dramatic change in approach by individual dairy companies, and development and launch of SmartSAMM. Pitched at farmers and their advisers, SmartSAMM aims to provide accessible tools and resources for all stakeholders, supported by training initiatives. Since 2010, the bulk milk SCC for Fonterra, representing around 90% of NZ suppliers, has dropped to 176,000 cells/ml, and cow SCC has dropped to 187,000 ells/ml in the 2015/16 season. The challenge remains to keep farmers and advisers engaged with achieving further improvements in milk quality, as well as reducing reliance on antibiotics.

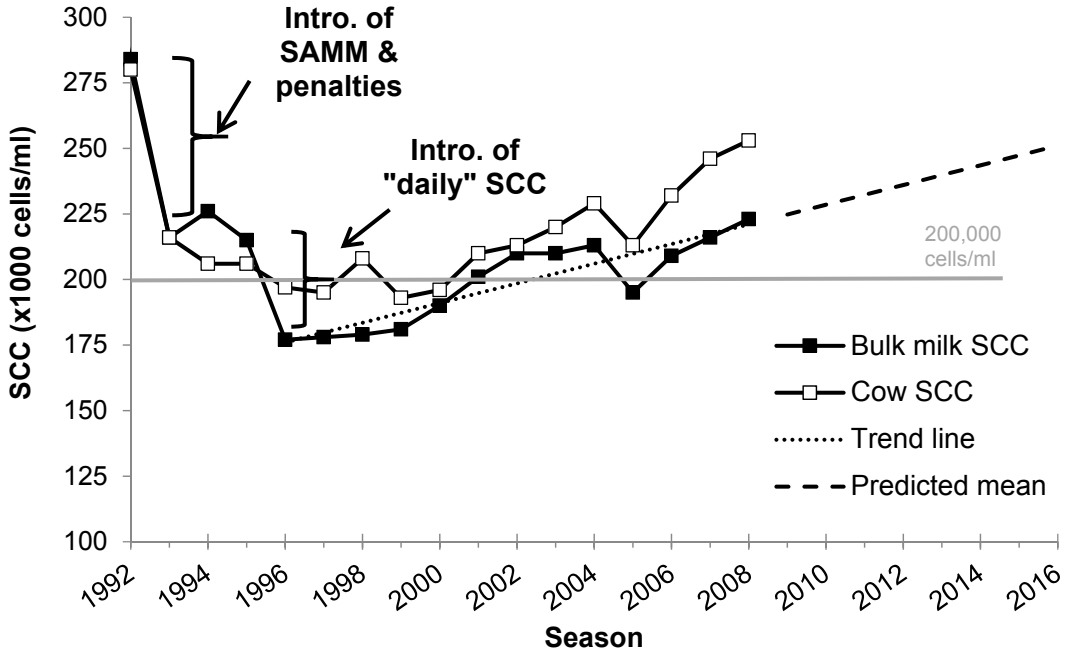
History of SAMM Plan
The Seasonal Approach to Managing Mastitis Plan (SAMM Plan) was first released in New Zealand in 1993 (Woolford et al. 1995). Based on the original UK 5-point plan, SAMM was designed to provide practical recommendations about control of mastitis, in alignment with the New Zealand seasonal calving system. Conceived by Professor Larry Smith, Mel Eden, and the late Dr. Murray Woolford (Joe 1993; Paine 1997), it proved to be highly successful (Lacy-Hulbert 1998), and formed a foundation for Australia's very comprehensive extension program, Countdown Downunder.

SAMM Plan used a consensus approach to package technical information into a concise, simple and easy-to-remember fashion. By gaining consensus across a small team of leading industry experts, it ensured consistency of message across vets and industry advisers (Paine 1997). The concise nature of the information ensured that farmers needed only to focus their efforts on a few key activities during each period of the year (Woolford et al. 1995). Technical content was reviewed annually and booklets distributed to all dairy farmers, via the dairy companies. SAMM was supported by regular articles in dairy farming magazines (Hook and Eden 2008), and industry Milk Quality Conferences hosted by dairy companies, or latterly by NMAC (National Mastitis Advisory Committee).

Release of the first SAMM Plan coincided with the introduction of the EU non-tariff barriers, requiring that bulk milk over 400,000 cells/ml be excluded from supply, as well as financial penalties applied by dairy companies on raw milk with a bulk milk SCC exceeding 400,000 cells/ml (Lacy-Hulbert 1998). Within 12 months, the SCC for the largest milk processor, New Zealand Dairy Group (NZDG), had dropped by more than 20% and averaged 216,000 cells/ml in

1993/94. But success was relatively short-lived and SCC improvements plateaued over the next 3 years (Figure 1).

Figure 1. Changes in annual average bulk milk SCC for New Zealand Dairy Group (1992 to 2000) and Fonterra (2001 to 2008; approx. 90-95% of suppliers), and annual average cow SCC determined from herd test information (approx. 70-75% of herds). Trend line and predicted mean shown for bulk milk SCC only. Notes relate to introduction of activities associated with SCC management.



In 1996, NZDG introduced per consignment testing of bulk milk SCC and penalties from the first day of supply. More frequent testing re-focused farmers' attention on mastitis control, and within a few months, further improvements in bulk milk SCC were achieved. But despite an 18% decline in bulk milk SCC during the first year of per consignment testing, very little improvements in cow SCC were observed (Figure 1), suggesting that farmers were actively managing the bulk milk SCC, by withholding cows from supply, but not making significant gains in mastitis control.

By 2008, much of the improvements achieved since the launch of SAMM had been reversed, and this coincided with significant changes in the industry.

Industry Change

Over the 10 years following release of SAMM, the national herd grew by a third (Table 1), average herd sizes grew by over 60%, from 193 to 315 cows, and milk production per cow increased by 20% (LIC 2008). At the same time, attention shifted away from milk quality as 14 dairy companies in 1993 gradually amalgamated to three, and Fonterra was established in 2000/01. This company is now responsible for collecting milk from around 85% of the 11,700 dairy farms in NZ.

Farm systems intensified to capitalize on the higher milk prices and counteract the rising cost of land. But, when adjusted for inflation, income per unit of milksolids only improved by around 10%, despite wide fluctuations in milk prices (Table 1). Advisers noted that the fluctuating milk prices impacted on milk quality. High prices softened the impact of economic penalties for poor quality milk, whereas low milk prices tended to reduce sales of dry cow antibiotic therapy and teat disinfectants.

Table 1. Summary of changes in the NZ dairy industry since 1994, from herd improvement statistics (LIC 2008; LIC 2016).

	1994/95	2004/05	2014/15	% change (20 years)
Total cows (million cows)	2.831	3.868	5.018	77%
Total effective area (million Ha)	1.176	1.412	1.746	48%
Cows per hectare	2.41	2.74	2.87	19%
Cows per herd	193	315	419	117%
% of herds' herd-tested	85.0%	75.8%	72.9%	-14%
% of cows' herd-tested	87.4%	72.7%	72.8%	-17%
Milk per cow (litres)	3,253	3,812	4,379	35%
Milksolids (MS) per cow (kg)	272	321	378	39%
Somatic cell count (x1000 cells/ml)	206	229	182	-12%
Average milk price (NZ$/kg MS)	3.40	4.58	4.69	38%
Av. milk price, inflation adj.	5.15	5.74	4.71	-8%

Much of the growth occurred in the South Island, in areas and communities more familiar with sheep and beef production. New personnel were attracted into the dairy industry, often with minimal skills and experience at detecting and controlling mastitis. Veterinarians around the country reported establishment of new herds, often using older, "budget" cows, with unknown mastitis histories, leading to contagious mastitis outbreaks.

During this time the annual average bulk milk SCC rose from 206,000 to 229,000 cells/ml and all signs were that this trend was set to continue. To remain competitive, a fresh approach was needed to convey the principles of SAMM to a new generation of milk suppliers, and help New Zealand farmers produce high quality milk at low cost.

Need for Change

In 2008, dairy processors recognized a need for change. Dairy processors such as Open Country and Tatua, who were targeting high-value, niche products, applied lower penalty thresholds for SCC (300,000 or 350,000 cells/ml) and/or provision of premiums for low SCC milk (Lodge 2008; Thomson 2008).

Fonterra, being the largest dairy processor and producing high volume commodity products, selected the farmer education pathway, reasoning that most of the benefits of low SCC milk would be captured inside the farm-gate by their suppliers. Several training courses were developed to improve the milking skills and milk quality knowledge of farm staff (Andela 2008).

Farmer focus workshops identified that farmers were increasingly frustrated with the costs and hassles of dealing with clinical mastitis, and attributed much of the risk to poor skills and performance by farm staff (McLeod 2008). Ironically, they also considered themselves responsible for training of their own farm staff. Coupled with a general lack of understanding around the basics of mastitis control, it was recognized that a new extension approach was needed to reach and educate the whole farm team, both on and off the farm.

Foundations for SmartSAMM

Against this backdrop of growth and intensification, the simplicity of SAMM became its drawback. Many farmers turned to their veterinarian as the primary source of knowledge (Cuthbert 2008) but SAMM did not provide sufficient technical information for veterinarians to trouble-shoot and solve intractable problems. With few benchmarks to gauge success for an individual farm, SAMM began to lose its appeal.

SmartSAMM, the Smart Approach to Minimising Mastitis, was developed by DairyNZ between 2010 and 2012. With support from NMAC, the process involved representatives from many organizations, including dairy companies, veterinarians, researchers, milking machine suppliers and testers, animal health suppliers and industry regulators.

Guiding principles included:

1. Involve technical experts, such as vets, milking machine technicians and dairy chemical suppliers in development of new resources, to promote engagement and ongoing support.
2. Provide consistent messages across different sectors of the industry, facilitated by easily accessible tools and resources.
3. Use web-based resources to enable assimilation of new findings and changes in regulations.
4. Support a process of continuous improvement, facilitated by vets and other advisers, and recognizing the value of the whole team, both on and off the farm.
5. Retain connection with the trusted SAMM brand, but differentiate, to emphasize a change in focus.

This development approach was strongly influenced by Dairy Australia's Countdown program, and findings from the Dutch Udder Health Centre (UGCN). Both programs placed a strong emphasis on a team approach to reduce mastitis and milk quality on individual farms (Penry et al. 2008; Penry et al. 2011), with veterinarians considered key to influencing farmers and facilitating change (Kuiper et al. 2005).

Continuous Improvement Process

An important role of SmartSAMM has been to promote the paradigm change in mind-set required to achieve sustained improvements in mastitis control and milk quality, facilitated through a continuous improvement process. This process is based on the principles first developed by Dr. W. E. Deming (Anon 2016), working with the Japanese manufacturing industry in the 1950s.

SmartSAMM has developed innovative tools and resources that help farmers and their advisers use continuous improvement to develop customized solutions. Called the SmartSAMM 4-step

process (Figure 2), it provides a central framework for the SmartSAMM programme that involves, but is not restricted to:

1. Assess performance using:
   Mastitis Focus Report
   Mastitis Investigation Kit

2. Identify scope for improvement using:
   SmartSAMM Gap Calculator

3. Review options using:
   Healthy Udder
   SmartSAMM Guidelines for farmers
   SmartSAMM Technotes for advisers

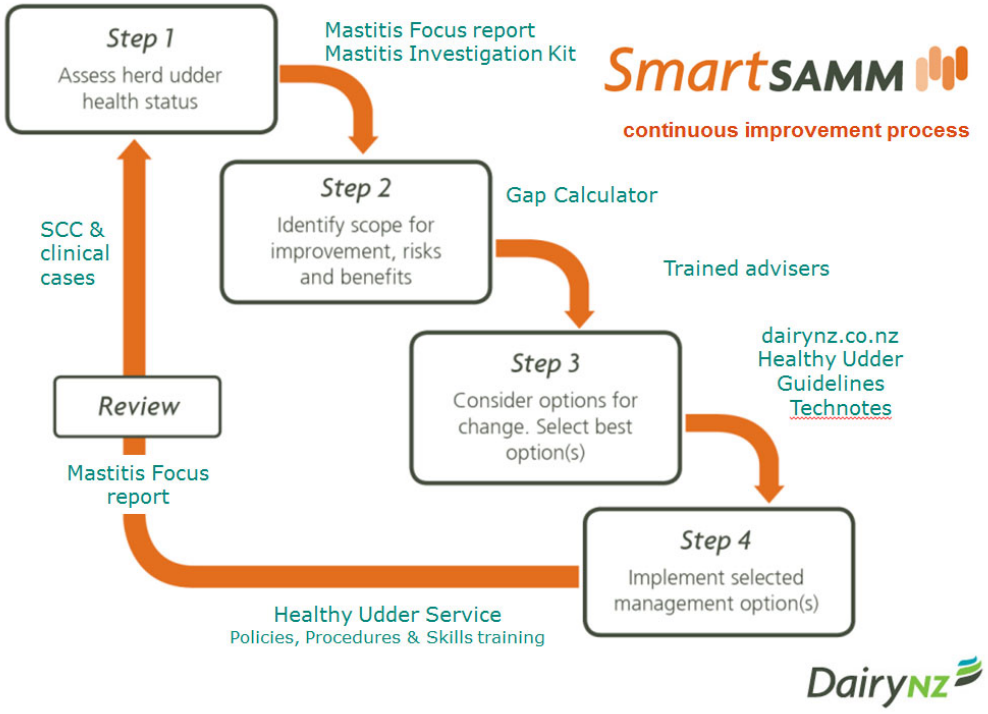
4. Implement a plan using:
   Healthy Udder Service.

Healthy Udder
The first innovation, "Healthy Udder" was released in 2011 to more than 11,000 dairy herds. Using simple words and many pictures, Healthy Udder condensed key mastitis control processes into three approaches: Prevent, Find and Treat mastitis. The document was printed on waterproof material and is sufficiently robust to be kept in the farm dairy, within easy reach when milking cows, or diagnosing and treating mastitis.

Designed for reference by people in the farm dairy, Healthy Udder has been used to train new people into the industry as well as develop skills of existing milkers, through informal training led by herd managers, vets and other advisers.

In 2013 and 14, the SmartSAMM team worked with key veterinarians to develop Healthy Udder Service, for use with farmer clients to help embed Healthy Udder practices into farm policies, procedures, and skills training.

Figure 2. SmartSAMM tools and resources that support the continuous improvement process.



SmartSAMM Gap Calculator

Online delivery of the SmartSAMM Gap Calculator followed in 2012. Using national and international data, the Calculator helps herd owners assess the impact of clinical and subclinical mastitis on production loss, risk of culling and treatment costs. The Gap Calculator uses a logarithmic relationship between BMSCC and production loss, assuming a 2.1% reduction in milksolids production for every doubling of the BMSCC above 100,000 cells/mL (Winkelman et al. 2007).

The Calculator helps estimate the opportunities of improving udder health, rather than focusing solely on the direct costs of dealing with clinical mastitis. This enables advisers to raise awareness of the potential gains, and support implementation of mastitis control measures.

SmartSAMM Guidelines and Technotes

Release of the SmartSAMM Guidelines and Technotes occurred in 2012. Adapted for NZ from the excellent mastitis technical resources developed by Countdown, the Guidelines provided recommendations for farmers, based on a seasonal, pasture-based dairying system. This was supported by the SmartSAMM Technotes to provide more technical information for advisers, linked to the same seasonal recommendations.

This extensive knowledge base has been incorporated into training materials for farm teams and veterinarians, and are available to all involved with mastitis and milk quality control through publication on the DairyNZ website: dairynz.co.nz/mastitis.

Progress to Date

The SmartSAMM project has been underway for 6 years, with launch of a dedicated website in 2012 representing a key milestone. The primary focus in the first few years was training veterinarians and other advisers to ensure sufficient trained and motivated people were available across New Zealand to work with farmers.

More recently, the attention has turned to more direct communication approaches with farmers, incorporating Healthy Udder messages into stockmanship and milking-related training activities for farm teams. The aim has been to motivate farmers with differing needs and motivations, to capture the benefits of improved udder health for their farm businesses.

Throughout, SmartSAMM has received great support from dairy companies and training providers. Formal training courses for those entering the industry, as well as more advanced courses for those in managerial roles on farm, have been updated and aligned with SmartSAMM resources, to ensure consistency of message and approach, and increase uptake of mastitis control measures on-farm.

Of particular benefit has been the Mastitis Support Programme operated by Fonterra. Developed in 2011, this involves proactive engagement with suppliers who are producing non-compliant milk. Using an escalating scale of interventions, from direct phone calls and physical visits by milk quality consultants through to milking time investigations by NMAC-accredited veterinarians, dairy suppliers have been actively supported until milk quality improves. Incentives, in the form of reimbursement of financial penalties to offset the cost of professional assistance, are given. The NMAC-accredited veterinarians are also listed on the DairyNZ website, and suppliers are encouraged to seek professional help before financial penalties are incurred.

Since 2008, the national SCC statistics have shown a dramatic improvement (Figure 3) with the bulk milk SCC for Fonterra dropping by over 20% and the cow SCC dropping by over 25%. As expected, suppliers that were in the highest quartile of SCC in 2009/10 have made the largest improvements in SCC (Figure 4). But all other quartiles have either improved or maintained position.

SmartSAMM cannot claim all the credit, but it has been a catalyst for change. The increased focus on milk quality by processors and favourable economic conditions have contributed to significant improvements in milk quality. But there are significant challenges ahead.

Figure 3. Changes in annual average bulk milk somatic cell count (SCC) for Fonterra and annual average cow SCC determined from herd test information during release of Healthy Udder and other SmartSAMM resources.

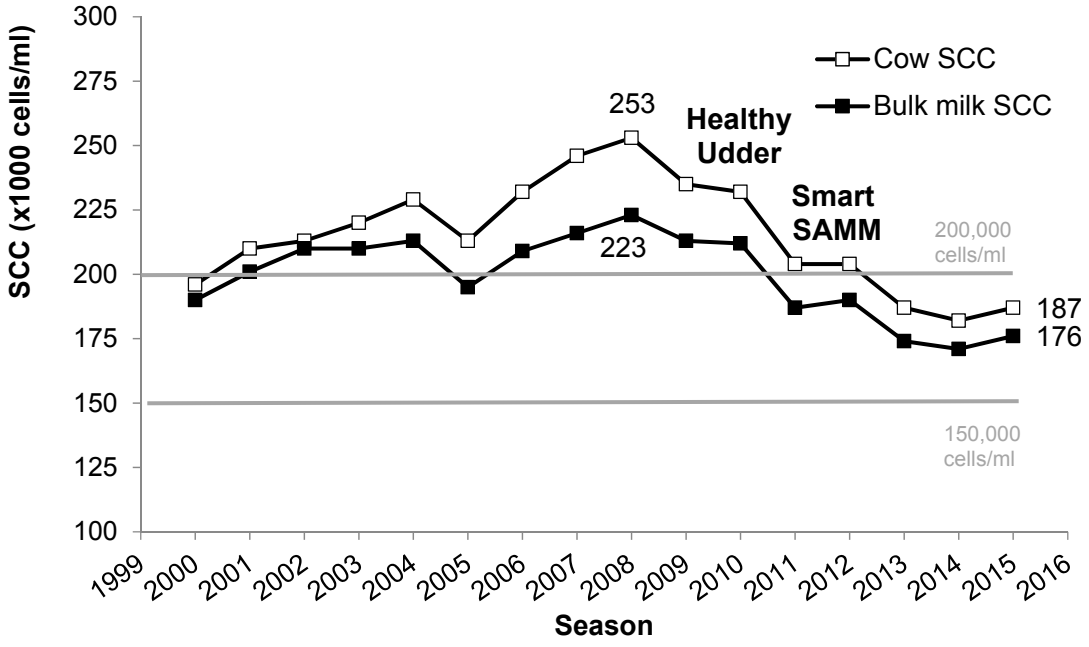
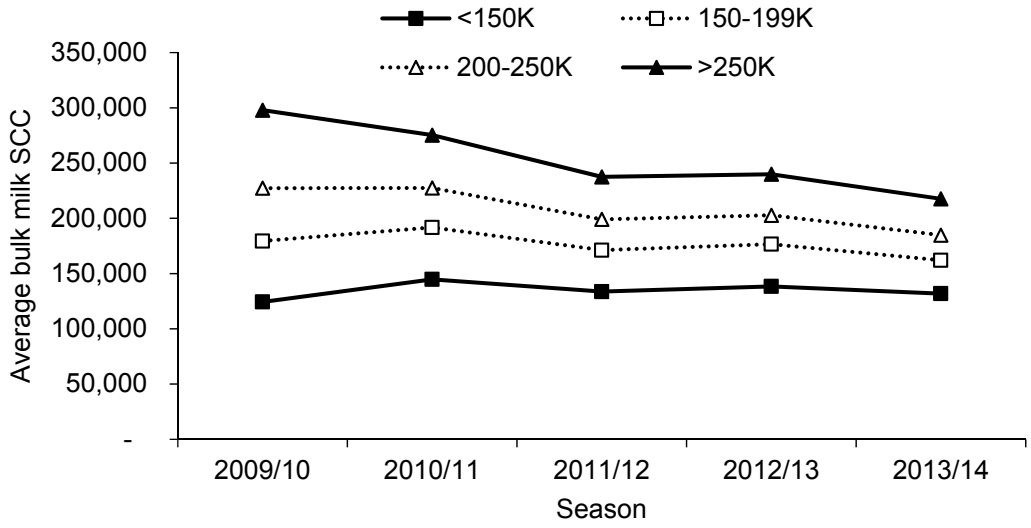


Figure 4 Changes in mean bulk milk somatic cell count (SCC; cells/ml) for herds supplying Fonterra (average 10,483 suppliers per season) by category of bulk milk SCC in the 2009/10 season.



Future Challenges

International and national concerns about antimicrobial resistance (AMR) are starting to influence management practices on farm. In the Netherlands, the veterinary profession implemented a raft of changes to antibiotic use in dairy cattle, including a total ban on prophylactic use of antibiotics since 2012 (Lam et al. 2014). In NZ, veterinary associations have signaled a ban on whole herd or prophylactic use of dry cow antibiotics by 2020 (NZVA 2016).

More robust decision-support tools are required to define risk factors for new infection across the lactation and the dry period, and clearly identify cows likely to be infected at the end of lactation, and hence eligible for dry cow therapy. Systems that allow better identification of likely pathogens, and hence probability of cure, are also required. The limitations of current culture techniques, and antimicrobial sensitivity testing, must be overcome, to support more prudent use of antimicrobials throughout the industry.

The SmartSAMM Mastitis Focus report is an innovative decision-support tool that has been made available to some herd managers through one herd improvement organization. But work is required to extend its reach. Developed by Dairy Australia, the Mastitis Focus is a powerful tool for helping farmers and their advisers monitor the profile of mastitis and milk quality measures in a herd, and provide key indicators that can be incorporated into mastitis control systems.

Many farms are making use of housing and stand-off systems to manage dry and early lactation cows during periods of inclement weather. Coupled with the feeding of supplementary feeds with a high energy content, these can change the types of bacteria in fecal matter, leading to a change in etiology of intramammary infections (Lacy-Hulbert et al. 2012).

Rapid changes in communication technologies also pose a challenge for extension programs, providing new materials that are engaging for mobile-savvy, image-hungry new entrants to the industry, often with English as a second language. The challenge for SmartSAMM is to remain relevant and accessible in this changing world, whilst supporting advisers and farmers to envisage new paradigms for mastitis management and milk quality on individual farms.

Conclusion

Mastitis management systems continue to evolve. Successful dairy farmers implement risk reduction systems, rather than reactive, problem-solving approaches. Changes in farm systems and prescribing approaches continue to extend these challenges. SmartSAMM must evolve too. In 2017, a refreshed version of Healthy Udder will be released to dairy farmers, to provide a tangible and visual reminder of the key principles mastitis control. This will be the first step in the next evolution of mastitis extension in New Zealand, to meet the needs of an ever changing industry.

References

Andela, R. 2008. Fonterra SCC penalty scheme. Proc. SAMM Milk Quality Conference. National Mastitis Advisory Committee, Rotorua, NZ, pp 124-126.

Anon. 2016. W. Edwards Deming. Accessed Dec. 2016 at: https://en.wikipedia.org/wiki/W._Edwards_Deming.

Cuthbert, S. 2008. DairyNZ Mastitis Management Perceptions Survey. Internal Report. Pages 1-37. LIC, Hamilton, NZ.

Hook, I., and M. Eden. 2008. History of SAMM and what is next? Proc. SAMM Milk Quality Conference. National Mastitis Advisory Committee, Rotorua, NZ, pp 162-167.

Kuiper, D., J. Jansen, R.J. Renes, C. Leeuwis, and H. van der Zwaag. 2005. Social factors related to mastitis control practices: The role of dairy farmers' knowledge, attitude, values, behaviour and networks. In: Mastitis in Dairy Production - Current knowledge and future solutions. Wageningen Academic Publishers, The Netherlands, pp 576-582.

Joe, A.K. 1993. The SAMM Plan. Proc. Ruakura Farmers Conference, Hamilton, NZ, pp 72-75.

Lacy-Hulbert, S.J. 1998. Impact of the SAMM plan on milk quality. Proc. NMC Annual Meeting, St Louis, MI, pp 28-34.

Lacy-Hulbert, J., J. Williamson, E. Kolver, H. Doohan, and J. Shelgren. 2012 Is coliform mastitis an emerging issue? Proc. New Zealand Milk Quality Conference, pp 7.06.1-7.06.7

Lam, T.G.J.M., C.G.M. Scherpenzeel, I.E.M. den Uijl, and G. van Schaik G. 2014. Dry cow therapy: Does it still deserve a blanket recommendation? Proc. NMC Annual Meeting pp 64-72.

LIC. 2008. New Zealand Dairy Statistics 2007/08. Pages 1-48. Hamilton, NZ.

LIC. 2016. New Zealand Dairy Statistics 2015/16. Pages 1-48. Hamilton, NZ

Lodge, S. 2008. Somatic cell count premiums - The New Zealand Dairies experience. Proc. SAMM Milk Quality Conference. National Mastitis Advisory Committee, Rotorua, NZ, pp 118-21.

McLeod, M. 2008. Report into the current knowledge and awareness of mastitis by NZ dairy farmers. Proc. Society of the Dairy Cattle Veterinarians of the NZVA. Palmerston North, NZ pp 171-175.

NZVA. 2016. NZVA position on DCT. Published by New Zealand Veterinary Association, Wellington. Accessed Dec. 2016 at http://amr.nzva.org.nz/resource-centre/policies-guidelines-and-reports.

Paine, M.S. 1997. Doing It Together: Technology as practice in the New Zealand dairy sector. Pages 1-222. Wageningen Agricultural University, The Netherlands.

Penry, J.F., P.B. Brightling, R.S. Dyson, and M.S. Paine. 2011 Developing new veterinary services in milk quality: A review of a recent mastitis risk management co-development in Australia. New Zealand Vet. J. 59:24.

Penry, J.F., R.S. Dyson, P. Brightling, and M. Paine. 2008. Countdown Downunder Max and Mastitis Focus: a new pathway in planned mastitis risk management from Australia's national mastitis and cell count program. In: *Mastitis control - From science to practice.* T. Lam, (ed.) Wageningen Academic Publishers, The Netherlands, pp 397-405.

Thomson, H. 2008. Improving milk quality. Proc. SAMM Milk Quality Conference. National Mastitis Advisory Committee, Rotorua, NZ, pp 106-115.

Winkelman, A.M. 2007. Effect of increased somatic cell count on lactation yields of milk, fat and protein. Proc. New Zealand Society of Animal Production, 67:293.

Woolford, M.W., I.S. Hook, M.T. Eden, and A.K. Joe. 1995. The “SAMM Plan” a Seasonal Approach to Managing Mastitis. Proc. Third IDF International Mastitis Seminar, Tel-Aviv, Israel. Section 4, pp 59-63.

A Strong Partnership among Farmers, Industry, and Customers: The Case of PDO Parmigiano Reggiano Cheese

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Parmigiano Reggiano (PR) is an extra hard (MFFB = 43-48), ripened, medium fat cheese. It is made, more or less in the same way, since the Middle Age. Historical evidences show that already in 13th-14th century Parmigiano Reggiano cheese had reached its typicality that is essentially unchanged until nowadays: the most known, even if not the oldest, is the one by Giovanni Boccaccio: "*And there was a mountain of grated Parmigiano cheese, on which people were making nothing but maccheroni and raviolis"* (*Decamerone*, 1351, description of the Bengodi land).

In 2015, 3.302.653 (132.800 tons) wheels have been done by 353 cheese factories using milk produced by about 245.000 cows reared in 3.272 farms in restricted production area.

The designation "Parmigiano Reggiano" is owned by the Consortium, founded in 1934 to protect the Designation of Origin and facilitate trade and consumption, promoting initiatives aimed to safeguarding the typicality and unique features of the product.

<u>The Parmigiano Reggiano Cheesemaking Technology</u>
PR cheesemaking technology is based on the use of raw milk from evening milking partially skimmed by overnight spontaneous creaming and mixed into copper vats with capacity of about 1.0 t with the whole milk from the morning milking.

A whey starter (a culture rich in indigenous thermophilic lactic acid bacteria, mainly *Lb. helveticus, Lb. delbrueckii ssp. lactis, Lb. fermentum* and *Strep. thermophilus* (Gatti 2014)) self-produced every day by every single dairy factory (industrial starters are not permitted) starting from the whey remaining from the previous cycle, is added to enrich the milk of positive microflora, lowering milk pH and so favoring milk coagulation. Milk coagulation is activated by calf rennet and curd is broken by a specific hand-tool called "spino".

Then, after a fast cooking of curd grains (the name "Grana" originates from the typical granular structure of the cheeses resulting from the cheesemaking conditions) at temperature of about 55-56°C, cooked curd remains in hot whey for about 60 min draining off the whey. Proper management of these operations contributes to create a habitat that facilitates the growth of raw milk and whey starter microflora.

Cooking of curd grains induces a sharp increase in total solids content of the curd from 21.6 to 37.5% and the rest in hot whey further contributes to drainage, increasing total solids content to 55.5%.

The curd is then placed in a plastic stenciling mold with a linen cloth on the first day (during which the temperature slowly decreases, remaining many hours above 50°C) to facilitate whey drainage. On the following day(s), a finely pierced steel mold is used.

The many hours spent at a temperature higher than 50 °C are managed as a CCP in HACCP plans and guarantee, together with many other factors (like pH, Aw, long aging, ...) the safety of PR cheese. Within 48 h from molding, lactose and galactose are fermented by thermophilic flora.

The cheese is then salted, for a length of time that depends on the size of the cheese, by dipping into saturated brines at room temperature for 15-20 days.

Wheels are then ripened at least until the age of 12 months in warehouses at temperature and relative humidity controlled to drive moisture loss to values of 29-31%. In this period the average a_w value falls to 0.92 at month 12 and a relevant process of proteolysis happens releasing smaller and smaller nitrogen compounds (Sforza 2012) strongly involved in sensory and nutritional features of aged product, so that at the age of 24 months around 25% of protein is present in form of free amino acids.

PR cheese is produced without the use of any additives.

The P.D.O. System

A tool to inform and guarantee the customers

Since 1996 Parmigiano Reggiano is a Protected Designation of Origin (PDO) cheese: the PDO system was born in UE in the 90's to protect typical denominations from frauds, strictly limiting the possibility of the use of the name to those producers who demonstrated the real, previous ownership of it.

So the PDO system wants to protect the producers but also, or better, first of all, the consumers that have to be sure of the authenticity of the product they are buying and informed about the way it is made.

This is why the production process is precisely described in a public, official document (www.parmigianoreggiano.com/consortium/rules_regulation_2/default.aspx) and a reliable system of control (to which every farm and every factory is submitted) is implemented.

Controls are performed by third independent body (paid by the producers but) operating under the control of the Ministry for Agriculture, Food and Forestry Policies. Third independent body is accredited by ACCREDIA (the body appointed by the Italian Government as the National Accreditation Body) either by activities inside dairy farms and factories (in order to verify the respect of the regulations during the production process) or by analyses on the product on the market in order to check that a cheese sold as "Parmigiano Reggiano" is a genuine Parmigiano Reggiano. Among the others parameters, absence of lysozyme and cyclopropanic fatty acids (markers of silages) and conformity of the isotopic and Mass Spectrometry profiles are checked (Caligiani 2016; Camin 2012; Popping 2016).

So transparency and reliability are the basics of the PDO system, and this is why it is now an important tool to connect and communicate with consumers to create the extra price linked to the perception they have of the quality of the product but also to the amount and the reliability of the information they can get about it.

Not only in Europe, customers are more and more interested in getting information referring not only and merely to the product but also to the process: the same term of "quality" is nowadays relevant to intrinsic (organoleptic, nutritional, …) properties of the product but also to the process from which the product is obtained, and often the features of the process that are requested are not directly appreciated because they determine in the product a better "objective" quality but because they are "subjectively" considered important according to cultural, ethic and so on reasons.

Of course PR regulation doesn't state and describe everything about the process but only the main, fundamental issues (many further certifications about animal welfare, GMO, organic, Kosher/Halal, and others are possible) but since some decades it tries to tell the customer how the PR producers work.

A tool to strengthen the partnership between industry and farmers
The price of the milk that is used to produce PR cheese is usually higher than the average market price, because the PR cheese price is usually high: this is due to the product quality, and to the reputation of the product but also has to be connected to the heavier production costs to produce a milk fit to be transformed in PR cheese.

For the dairy factories that produce PR cheese is fundamental to be confident about the respect of the rules by the farmers which provide the milk: these rules are written in the regulation, that so is a proper tool to regulate the partnership between industry and farmers since it states in a clear and binding way the essential characteristics of milk production process.

The main obligations that the farmers are obliged to respect are about:
- milk production area: only in Parma, Reggio Emilia, Modena and part of Bologna and Mantova provinces;
- cows feeding: no silages, by-products and added fats can be used. The fodder has to be produced in the production area described before and constitute at least the 50% of dry matter ingested by the cows. The ban on the use of silages allows not to use preservatives (like lysozyme) in cheese production, that is probably the most relevant feature of PR process. In fact, only milk with clostridia spore near zero (that means less than 100 cells/liter!) is fit to be used to produce hard cheeses without neither preservatives nor physical treatments. It has been documented that mesophilic lactic flora coming from the milk (and not from the starter) survive in the aged cheese (Gatti 2008) and is responsible for the specific proteolytic process (De Dea Lindner 2008) involved in organoleptic and nutritional features of the product. So the use of hay or grass instead of silages, creating a completely different ruminal and intestinal environment, strongly contribute to characterize the milk fit to be used in PR production;
- cows milking: the milk has to be delivered to cheese factories twice a day within two hours from the end of every milking, in order to permit to keep it at temperature higher than 18°C and so preserving mesophilic flora and coagulating properties of casein.

Considering the absolute necessity of the respect of these (and many other) rules, it is clear that a strong, well defined partnership between farmers and industry is important: the .P.D.O. Regulation is the core of this partnership, also based on the cooperative structure of the most part of the dairy factories.

Conclusions

Parmigiano Reggiano cheese is done, more or less in the same way, since the Middle Age and is now a Protected Designation of Origin (PDO) cheese. PDO system was born in UE in the 90's to protect typical denominations from frauds. Nevertheless, with time, it has become a tool to inform and guarantee the customers about what and how the industry does to produce the cheese they buy, so it is now a way to connect and communicate with consumers in order to create the extra price linked to the perception they have of the quality of the product but also, more and more important, of the production process.
It also strengthens the partnership between industry and farmers since it states in a clear way the essential characteristics of milk production process.

References

Caligiani, A., M. Nocetti, V. Lolli, A. Marseglia, and G. Palla. 2016. Development of a Quantitative GC–MS Method for the Detection of Cyclopropane Fatty Acids in Cheese as New Molecular Markers for Parmigiano Reggiano Authentication. J. Agric. Food Chem. 64 (20):4158.

Camin, F., R. Wehrens, D.Bertoldi, L. Bontempo, L. Ziller, M. Perini, G. Nicolini, M. Nocetti, and R. Larcher. 2012. H, C, N and S stable isotopes and mineral profiles to objectively guarantee the authenticity of grated hard cheeses. Analytica Chimica Acta 711:54.

De Dea Lindner, J., V. Bernini, A. De Lorentiis, A Pecorari, E. Neviani, and M. Gatti. (2008). Parmigiano Reggiano cheese: evolution of cultivable and total lactic microflora and peptidase activities during manufacture and ripening. Dairy Sci. Technol. 88:511.

Gatti, M., B. Bottari, C. Lazzi, E. Neviani, and G. Mucchetti. 2014. Microbial evolution in raw-milk, long-ripened cheeses produced using undefined natural whey starters. J. Dairy Sci. 97:573–591.

Gatti, M., J. De Dea Lindner, A. De Lorentiis, B. Bottari, M. Santarelli, V. Bernini, and E. Neviani. 2008. Dynamics of Whole and Lysed Bacterial Cells during Parmigiano-Reggiano Cheese Production and Ripening. Appl. Environ. Microbiol. 74:6161.

Popping, B., E. De Dominicis, M. Dante, and M. Nocetti. 2016. Identification of geographic origin of Parmigiano Reggiano (PDO) Cheeses deploying Non-Targeted Mass Spectrometry and Chemometrics. Foods, submitted.

Sforza S, V. Cavatorta, F. Lambertini, G. Galaverna, A. Dossena, and R. Marchelli. 2012. Cheese peptidomics: a detailed study on the evolution of the oligopeptide fraction in Parmigiano-Reggiano cheese from curd to 24 months of aging. J. Dairy Sci. 95:3514.

Genomic Information to Improve Milk Quality in Dairy Cattle

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Introduction

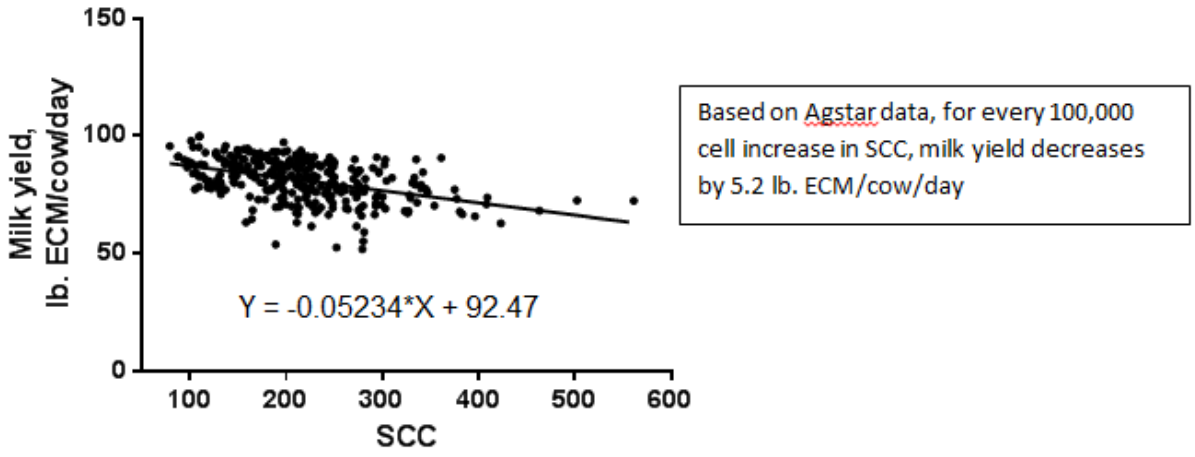
Genetic evaluation and selection in dairy cattle has largely focused on production traits such as milk and protein production for many years. Genetic improvement causes an average U.S. Holstein cow born in 2014 to produce over 14,000 pounds more milk in one lactation than her ancestors born in 1960. This increase in milk yield from genetic selection may be accompanied by correlated increases in genetic susceptibility to clinical mastitis and somatic cells. In response, Dairy researchers (USDA/AIPL) and cattle breeders responded by developing national genetic evaluation programs for Linear Somatic Cell Score (SCS) in 1994. The availability of these SCS evaluation and the incorporation of them into multi-trait indexes has helped U.S. dairy breeders reverse the trend and reduced the national herd average test-day Somatic Cell Count from 319 in 2003 to 199 cells/ml, 1000's in 2013 (Norman and Walton 2013). Continued enhancements of these predictions and the incorporation genomic information today presents an unprecedented opportunity to improve milk quality using genetic and genomic information.

Key Reasons to Improve Mastitis through Genetic and Genomic Selection

High Cost and High Prevalence

Mastitis is an incredibly expensive disease, robbing herds of pounds of production, days of productive life, and reproductive performance. Milk quality should be managed not only to obtain a premium, but also to manage the dramatic negative effect both clinical and non-clinical mastitis have on farm profitability. In a study conducted by Zoetis and a financial consulting services partner, they determine that as somatic cell count (SCC) increased, Energy Corrected Milk (EMC) yield (Figure 1) and 21-day pregnancy risk decreased, while death losses and days open increased. The analysis showed that management of SCC has little to do with revenue generation associated with a quality bonus; rather, it affected production, death loss, veterinary costs, and reproductive performance. Additionally, mastitis is a common disease affecting dairy cattle. A recent study (Vukasinovic et al. 2017) observed an average prevalence of recorded clinical mastitis at 25% in more than 4 million lactation records analyzed in the United States.

Figure 1. Regression between SCC and Energy Corrected Milk yield (Zoetis Technical Bulletin, 2016)



Genetic Correlation with Production and other important traits

It has been broadly documented the positive genetic correlation between mastitis susceptibility and milk production (Heringstad et al., 2000). A recent study shows approximated genetic correlations as well as Pearson (product-moment) correlations between Mastitis trait predictions and official gPTA for milk production in the national genetic evaluation (Vukasinovic et al. 2017). These authors indicates that mastitis and milk yield showed a positive correlation of 0.25, indicating that animals with higher genetic potential for yield were more susceptible to Mastitis. Mastitis is also known to be highly correlated with SCS, having an approximated genetic correlation of 0.72 (Vukasinovic et al. 2017); an identical result was reported by Boichard and Rupp (2000). Importantly, this relationships show that if mastitis is ignored in breeders selection objectives, the large relative weight traditionally placed on milk production is expected to decrease mastitis resistance (Heringstad et al., 2000). Additionally, SCS and mastitis are negative correlated with several other economic important traits such us Productive Life, Daughter Pregnancy Rate, Cow and Heifer Conception (Table 1; VanRaden and Cole, 2014; Vukasinovic et al. 2017). Therefore, the inclusion of mastitis or SCS traits in breeding programs is needed to neutralize the undesirable correlated responses (Heringstad et al., 2000).

Table 1. Genetic correlations among SCS and other economically important traits (VanRaden and Cole, 2014)

	Productive Life	Daughter Pregnancy Rate	Cow Conception Rate	Heifer Conception Rate
Genetic	-0.45	-0.27	-0.25	-0.12
Phenotypic	-0.40	-0.05	-0.20	-0.04

Availability of Economic Selection Indexes and Genomic Predictions
Selection indexes are a critical component of many selection strategies as they provide a path for dairy producers to select for comprehensive genetic improvement across a host of traits.

The use of economic selection indexes helps to ensure that the distribution of selection pressure applied to component traits is appropriately balanced relative to the economic impact of the individual traits on dairy profitability. For instance, breeders can select of mastitis resistance and at the same time for production, fertility, type, longevity, etc. Examples of these economic selection indexes are Net Merit (NM$), Dairy Wellness Profit (DWP$™), Total Performance Index (TPI).

Additionally, the use of genomics provides predictions of genetic merit with greater reliability than could be sensibly achieved using traditional progeny-based evaluation systems, especially in young animals. This is particularly relevant for lowly heritable traits where genomic predictions can provide more information than could ever be achieved in the lifetime of a typical cow. Recalling the results presented in Table 2, a prediction for somatic cell score in a young female with reliability of 73% would be equivalent to more than 75 daughters in a traditional genetic evaluation (CDCB 2016). Few dairy cows will ever produce that many daughters and yet this degree of reliability is achievable with genomic testing. Therefore, the increased reliabilities and shortened generation interval from using genomic evaluations are the most important factor in increasing the rate of genetic improvement in dairy breeding.

Table 2. Comparison of December 2016 genomic and traditional evaluations (n= 731,443 Holstein heifers; CDCB 2016)

	Reliability (%)			
Traits	**Genomic average**	**Traditional average**	**Difference[1]**	**Genomic DE[2]**
Net merit ($)	72	26	46	42.9
Milk (pounds)	75	28	48	32.5
Fat (pounds)	75	28	48	32.5
Protein (pounds)	75	28	47	33
Productive life (months)	71	24	47	102.7
Somatic cell score	73	25	47	75
Daughter pregnancy rate (%)	68	22	46	184.8
Final score	73	23	50	31.5
Sire calving ease	57	27	30	44.6
Daughter calving ease	54	23	31	52.7

[1]Genomic minus traditional.
[2]DE = daughter equivalents (the number of daughters with records that the genomic information is worth).

In summary, the incorporation of genomics in the evaluation of dairy cattle genetics is giving to the U.S. dairy industry a good base of knowledge for informed decisions.

Genetic Evaluation and Selection for Milk Quality (USA evaluations)

Direct selection for clinical mastitis

Direct selection for Mastitis can be performed using linear (normal distributed) or threshold models (binary data). In a recent publication, Vukasinovic et al. 2017 reported the development of genetic and genomic evaluation for mastitis and other wellness traits in U.S. Holstein cows. They analyze the mastitis trait separately using a univariate threshold animal model with repeated observations reporting a heritability of 6.9%. Mastitis data were obtained from approximately 240 herds located in 29 different states in all regions of the United States. Each herd provided information on 13,720 animals, on average. As of January 2016, over 3 million health events including mastitis from approximately 14.5 million lactation records have been collected for the analysis (Vukasinovic et al. 2017). These predictions for the Genetic Evaluation of Wellness Traits in the United States are then expressed as genomic standardized transmitting abilities (STA), similar to how type traits are expressed. Values are centered at 100 with a standard deviation of 5. For mastitis predictions, a value of 100 represents average expected mastitis risk and values of greater than 100 reflect animals with lower expected average disease risk relative to herdmates with lower STA values. Higher values are more desirable for mastitis traits, thus selecting for a high STA will apply selection pressure for reduced risk of mastitis.

For the genetic evaluation of wellness traits in the United States, mastitis is defined as a binary event—having a value of 1 if a cow has been recorded with the disease at any point during the lactation and zero otherwise—regardless of how many times disease incidence or treatment was actually recorded during the lactation. Each animal was required to have a lactation record with a valid calving date and a lactation number as well as a calving interval between 250 and 999 d (Vukasinovic et al. 2017).

Indirect selection using somatic cell counts

Somatic Cell Score (SCS): Predicted Transmitting Abilities (PTAs) for SCS are indirect predictors of susceptibility to mastitis. Lower values indicate a more favorable somatic cell value throughout the duration of a cow's lactation compared to the breed base.

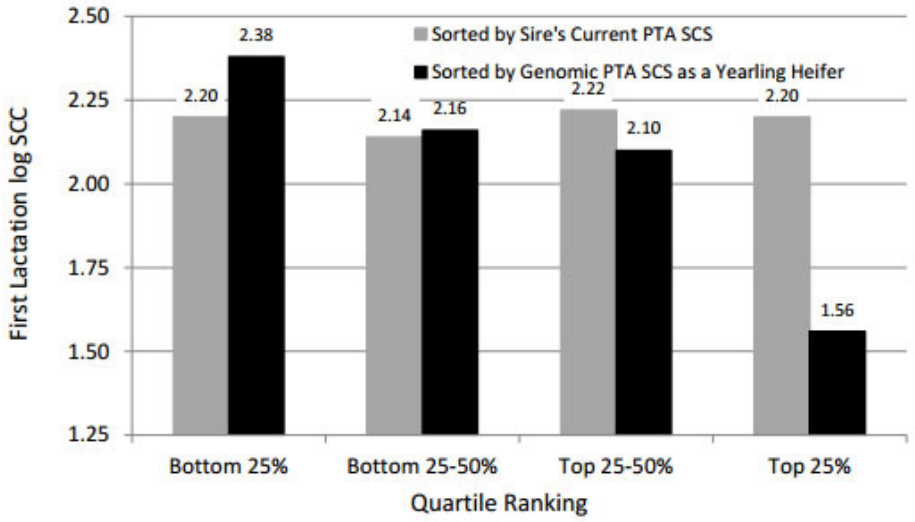
The genetic evaluation of the Council on Dairy Cattle Breeding (CDCB) utilizes somatic cell counts (SCC) data from milk samples collected on test day. These counts, ranging from a few thousand to 10 million somatic cells per milliliter of milk, are converted to "log equivalents" (log base 2 (SCC / 100,000) + 3) and averaged for each test day in the first 305 days of lactation (Cassell 2009). Additionally, this trait PTA for each animal is scaled by adding 3.00. The heritability for SCS trait is 0.12, one of highest heritabilities for any of the health related traits.

Associations between SCS, Mastitis Traits, and Milk Quality Outcomes

A fresh approach to demonstrate the value of this information and gain farmers and veterinarian's confidence in these mastitis predictions is to determine the relationship between genomic predictions and observed performance of the evaluated animals (Weigel et al., 2015). Weigel et al., 2015 compared early genomic predictions for SCS with their subsequent lactation performance in Holstein cows beyond 60 days in milk. The analysis was based on sorting heifers into quartiles based on their own genomic PTA for SCS at 12 months of age and also sires' current SCS PTA values. These authors compared the genomic predictions for somatic cell score (SCS) with actual average monthly log somatic cell count (SCC) in first lactation for 216

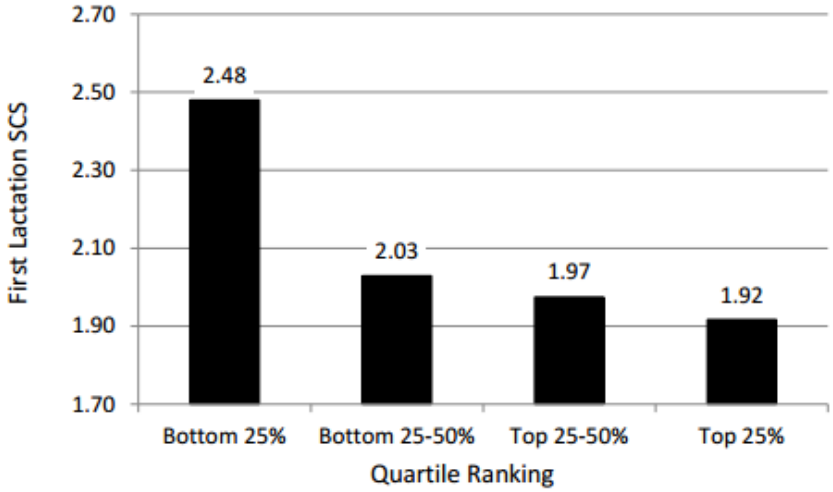
Holstein cows. As shown in Figure 2, there was a clear increase in first lactation log SCC when cows were sorted based on genomic PTA for SCS as a yearling heifer, and the difference between highest and lowest quartiles for genomic PTA (2.38 vs. 1.56) was much greater than for sire PTA (2.20 vs. 1.56).

Figure 2. Average log somatic cell count in first lactation for 216 Holstein cows in the Allenstein Dairy Herd at the University of Wisconsin-Madison, according to quartile for genomic PTA for somatic cell score at 12 months of age and quartile for sire's current PTA for somatic cell score (Weigel et al., 2015).



A second herd was analyzed using data from one of the top commercial dairies in Wisconsin, where genomic testing is part of the management routine for all heifer calves. This herd has 920 Holstein cows, with a rolling herd average of approximately 31,000 pounds on 3X milking. All animals with genomic predictions in August 2013 were used in the analysis, and the genomic predictions were compared with their subsequent first lactation performance (Figure 3; Weigel et al., 2015).

Figure 3. Average somatic cell score in first lactation for 192 Holstein cows in a leading commercial herd, according to quartile for genomic PTA for somatic cell score as a yearling heifer.

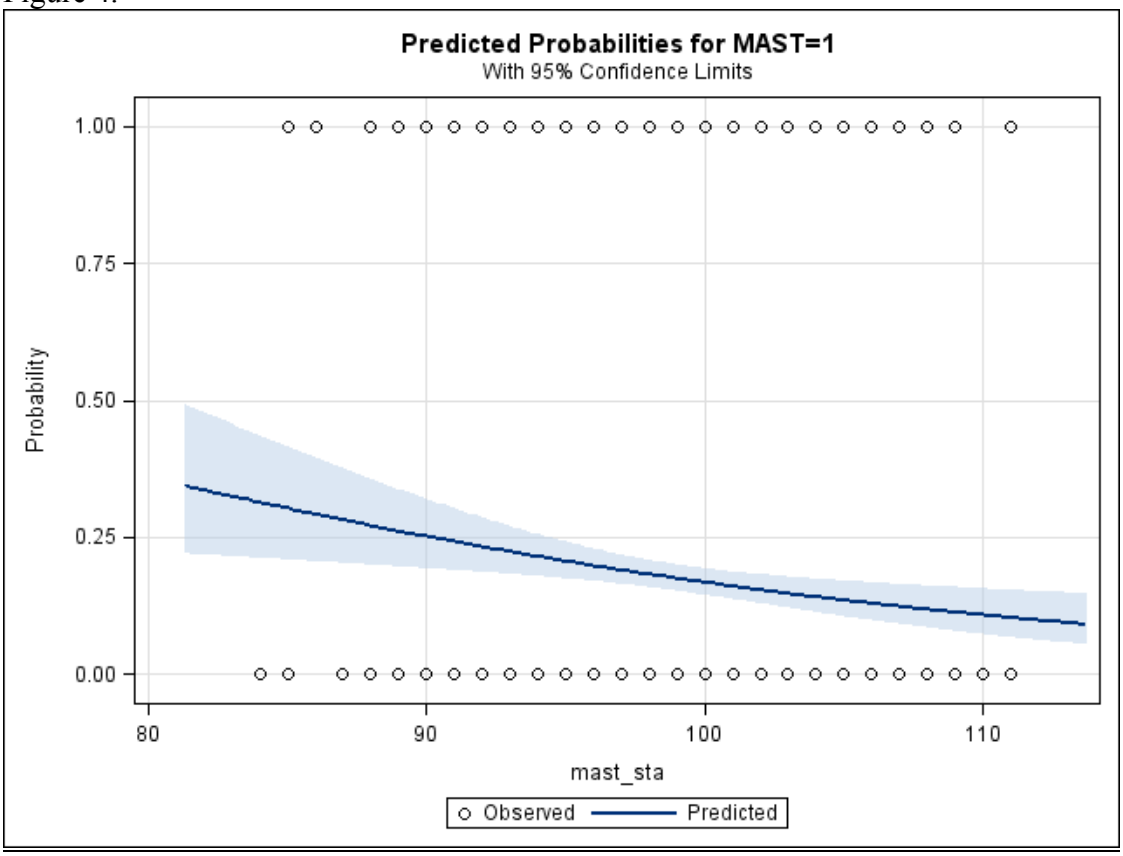


As shown in Figure 3, early genomic predictions of somatic cell score were quite effective for identifying heifers that were more likely to suffer from clinical or subclinical mastitis in the future, with average first lactation SCS of 2.48 for the cows with poorest genomic predictions and 2.03, 1.97, and 1.92 for the other three quartile groups (Weigel et al., 2015). Thus, genomics can also be used to identify heifers that are more likely to suffer from clinical or subclinical mastitis than their contemporaries once they enter the milking herd.

Similar results were obtained by Weigel et al 2016 (Data in File - Zoetis 2016) using a logistic regression, they quantified differences in mastitis prevalence per point of STA (Mastitis prediction) in the first lactation (n=975). Initially, herd, year and season where not included in the model to better visualize the relationship and associated 95% confidence intervals (Figure 4). Estimating the reduction in mastitis prevalence per point of STA demonstrates an appreciable relationship between the two metrics through 305 DIM (Figure 4). The relationships depicted in this figure indicate that there are appreciable decreases in mastitis prevalence as the Mastitis STA increases.

Figure 3 illustrates the expected phenotypic prevalence of recorded mastitis events per point of STA in the first lactation (Weigel et al, 2016; Data in File - Zoetis 2016).

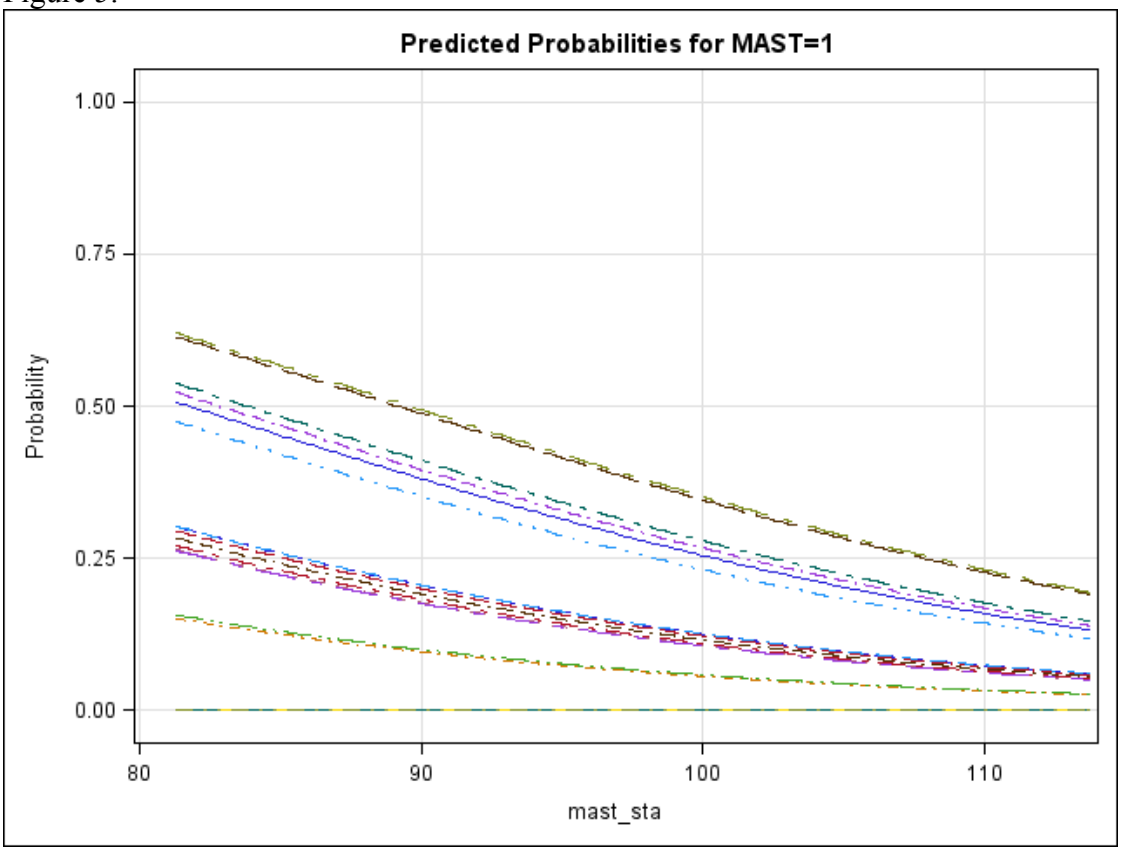
Figure 4.



Similarly, when the same logistic regression was run, but including herd, year and season in the model, the negative relationship is maintained with substantial variation across the herd year, and seasons (Figure 5). No surprisingly, variation is observed between herds, years and seasons given differences in management, technology and environments but the similar negative relationship is observed across herds, years and seasons with higher STA values associated with lower rates of mastitis.

Figure 5 illustrates the expected phenotypic prevalence of recorded mastitis events per point of STA in the first lactation including herd, year and season in the model as a fixed effect (Weigel et al 2016 - Data in File - Zoetis 2016).

Figure 5.



Using Genetic Evaluations for Mastitis and Udder Health

Comprehensive index like Net Merit and Dairy Wellness Profit are the best way to make economically sound selection decisions. The best way to use mastitis and udder health traits is through selection indexes that combine production, fertility, health, and wellness traits. Dairy Wellness Profit, Net Merit, or the related indexes (Breed Associations Indexes, i.e. TPI), are designed to identify AI bulls and cows that have optimum combinations of favorable genes for many traits for lifetime economic merit.

Take Home Messages

- Improving udder health through genetic selection presents a compelling opportunity for dairy producers to help manage mastitis prevalence and improve profitability when coupled with sound management practices
- The incorporation of genomics in the evaluation of dairy cattle genetics is giving to the U.S. dairy industry a good base of knowledge for informed decisions.
- Although mastitis and udder health are strongly influenced by the environment, the incorporation of SCS and Mastitis traits into the genetic evaluation followed by the notorious improvement in genetic trend is a clear evidence to support the influence of genetics on udder health

Association studies between genomic predictions and observed clinical mastitis indicate that genomic data of young calves and heifers can be used to effectively predict future udder health performance.

Selecting for udder health (Mastitis and SCS) can produce substantial changes in the mastitis prevalence. An effective method for selecting for improved milk quality is to incorporate the udder health traits (SCS, Mastitis Risk) with other economically important traits (production, Fertility) into a selection index such as Net Merit (NM$), Dairy Wellness Profit (DWP$™), Total Performance Index (TPI) or Jersey Performance Index (JPI) using genomically enhanced prediction (GPTAs).

References

Boichard, D., and R. Rupp. 2000. Phenotypic and genetic relationships between somatic cell counts and clinical mastitis in French dairy Holstein cows. Interbull Bull. 26:66–72.

Cassell, B. 2009. Sire Evaluations for Health and Fitness Traits – Virginia Cooperative Extension Virginia Tech. https://pubs.ext.vt.edu/404/404-087/404-087.html.

Council on Dairy Cattle Breeding. 2016. Comparison of December 2016 genomic and traditional evaluations. Accessed Dec. 23 of 2016. https://www.cdcb.us/eval/summary/comparexml_menu.cfm?R_menu=v_1612.v_Heifers.v_Holstein_wddx#StartBody.

Heringstad, B., G. Klemetsdal, and J. Ruane. Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries, Livestock Production Science, Volume 64, Issues 2–3, June 2000, Pages 95-106, ISSN 0301-6226. http://dx.doi.org/10.1016/S0301-6226(99)00128-1.

Norman and Walton. Somatic cell counts of milk from Dairy Herd Improvement herds during 2013. Council on Dairy Cattle Breeding, Beltsville, MD. https://www.cdcb.us/publish/dhi/dhi14/sccrpt.htm.

Schutz, M.M. 1994. Genetic evaluation of somatic cell scores for United States dairy cattle. J. Dairy Sci. 77:2113– 2129

VanRaden and Cole. CDCB. 2014. Net merit as a measure of lifetime profit: 2014 revision. Animal Improvement Program, Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA. http://aipl.arsusda.gov/reference/nmcalc-2014.htm.

Vukasinovic, N., N. Bacciu, C. A. Przybyla, P. Boddhireddy, and S. K. DeNise. 2017. Development of Genetic and Genomic Evaluation for Wellness Traits in U.S. Holstein Cows. Journal of Dairy Science, Volume 100 , Issue 1 , 428 - 438

Weigel, K.A., A. Ashley, and V.E. Cabrera. 2015. Effective Use of Genomics in Sire Selection and Replacement Heifer Management. In: Proc. Western Dairy Management Conference, Reno, NV.

Weigel, K., A. McNeel, and F. Di Croce. 2016. Zoetis Data in File. Zoetis Inc.

Zoetis Technical Bulletin. 2016. Identifying Drivers of Profitability on Commercial Dairies. Zoetis Inc.

Mastitis, Milk Quality, and Dairy Challenges in Developing Africa

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Introduction

Developing Africa does not have a history of dairy farming. The keeping of local indigenous cattle is important and a sign of wealth. These local cattle are hardy and resistant to many of the tick borne diseases which are commonplace. Dairy farming in developing Africa is not for the faint hearted, you need to have nerves of steel, grit and determination to hang in there and make it a success.

Africa poses a challenging climate with a rainy season for four months of the year and this can be very intermittent. Planted crops often fail as the expected rains do not arrive in time for germination and even then, if the rains do not continue, crops fail. Cows will eat a wide range of forages depending on the type of farmer. Concentrates may also be fed but the quality can be highly variable. Africa is the world's dumping ground for poor quality products and very high prices due to a range of tariffs and corruption in many countries.

Political instability is always there. You have no idea what law will be imposed next or when governments change the laws on tariffs or whatever. You need to be strong willed and brave to farm in Africa and then you will have years when crops fail.

Infrastructure and Support

Infrastructure is highly variable and they do not enjoy the same facilities that we take for granted in the Western world. Veterinarians are few and far between, supply of medicines is intermittent including vaccines for controlling important diseases like Rift Valley Fever, Lumpy Skin Disease and Foot and Mouth Disease. This puts stock at risk. Local cattle wander and can act as the vector for many of these diseases.

There are few extension services; but some are provided by local dairy associations, aid organisations or limited government services. Unfortunately, many of these staff are poorly trained and are using materials and information that are out of date. Finances means that they cannot get access to the wealth of information that we all take for granted from the likes of meetings like the NMC, Dairy Expo and other such gatherings to exchange ideas.

Climate

Climatic variation can be severe. In Mozambique in the rainy season you have very high humidity and temperatures of over 35C. Cows suffer from great heat stress. The corrals can become very muddy, you can have up to 150 mm or 6 inches of rain in a day at times, and so environmental clinical mastitis is a problem. In very wet weather cows can literally bury themselves in mud and manure to stay cool and the cow's immune system is under severe challenge.

However, a warm climate means that there can be two or three crops a year, especially if irrigated. Most farmers use maize silage as the basis of their forage. Some will make hay but the quality can be highly variable. Some have tried to make grass silage but this has proved unsuccessful. Maize silage is generally stored underground and harvesting is very labour intensive. Most maize harvesters cut one of two rows at a time.

Processing and Milk Testing

There are dairy processors with quality facilities for milk processing but they often struggle with the quality of the raw ingredients coming into their factories. Electricity supplies and a lack of bulk tank or other cooling facilities means that TBCs can be very high.

Accurate milk quality testing is uncommon. Many will rely on the CMT. There is some western cell count testing equipment but standard solutions for accurate calibration is rare and maintenance of this equipment will be variable. Test results can be very variable. There are no individual cow cell count testing services unless people buy their own equipment like the DeLaval DCC cell count tester, but even then, the availability and costs of the individual test cassettes are often twice that we pay in the western world.

Labour

Unfortunately, men in Africa are generally lazy and leave the women to do the work, run the home, work in the fields and rear the children. Men are complacent and expect that the women will provide.

Labour is in abundance and cheap. A litre of milk in Zimbabwe is sold to the dairy processor for about $0.50, well over 50% more than the world price, and the minimum daily wage is only $2.00/day. This shows the value of milk. A Zimbabwe farm worker gets paid the equivalent of 4 litres of milk a day.

Milk Value and Demographics

Milk is valued for its nutritional value especially for the young and old. Neonatal mortality is very commonplace and much of this is due to malnutrition. In Malawi about 50% of children are malnourished and there are significant problems with cognitive disorders. Milk helps bridge this gap.

The price of milk varies greatly. In Malawi it's $0.23/litre, in Zimbabwe is $0.55 and in Ethiopia $0.70 to $0.90/litre. The margin for Ethiopian farmers is $0.40 to $0.60/litre making dairying very profitable.

The variation in dairy cow numbers and production is shown above. Mozambique has a population of 22 million but only 4,000 dairy cows. Kenya has double the human population and has almost 4 million cows, almost 500 times more cows per hear of human population.

Yield per cow will vary according to nutritional intake. Small scale farmers might have cows producing 4 to 5 litres a day if they are just giving them some very poor quality forage while others will be over 20 litres a day by supplementing quality forage with concentrates.

Commercial farmers often average between 20 and 30 litres per day and some herds are producing even more.

Country	Population (million)	Number of dairy cows	Average yield per cow (litres)
Angola	13	19,500	1,000
Botswana	2	5,000	1,660
Burundi	10	890,000	22
Ethiopia	88	4,507,000	322
Kenya	40	3,800,000	1,050
Madagascar	21	535,000	6,944
Malawi	16	20,000	1,750
Mauritius	1	5,800	690
Mozambique	22	3,000	4,000
Namibia	2	2,900	7,069
Rwanda	11	1,200,000	292
South Africa	49	700,000	4,543
Swaziland	1	6,323	1,265
Tanzania	42	814,000	1,229
Uganda	33	1,600,000	625
Zambia	14	64,200	1,003
Zimbabwe	12	16,000	2,625

Small Scale Farmers

Farmers can be categorised according to herd size. Small scale farmers will tend to have between one and ten cows and will be hand milking. Hand milking poses a lot of potential problems. It is cheap and easy, no capital outlay, but the spread of Staph aureus infections is high. Cows flick bits of muck and mud into the bucket of milk. Scouring cows will contaminate even more! Milk is usually strained through gauze to take out much of the physical contamination.

Small scale farmers will have basic facilities and knowledge. Milk is sold locally as either fresh or sour milk, called ‘lacto’, which is very popular. Some of the small scale farmers will sell milk to larger milk buyers but the quality will be very variable. Cell counts and TBC are likely to be high. There will be no facility to cool milk and hygiene standards are likely to be poor.

Many of the small scale farmers keep their cows in a corral by their home. This is to avoid theft or wild animals attacking their stock. In Mozambique for example, the corral will be composed of wooden fences and have clean water and feed area and a race type system for milking. Feed is grown away from the house and is cut every day and brought back to feed the cows. This will require one labour unit. Water needs to be brought back from the well and this might be up to one kilometre away and with 100 to 125 litres of water needed per cow per day, this will be another labour unit. Surplus milk then has to be taken to the milk collection centre if not sold locally. As there is no cooling facility, this has to be taken twice a day, in some cases up to 2 kilometres. The transport choices are walking (very common), bicycle or local bus (very expensive). Walking is common and so you can have two labour units just taking surplus milk to the collection centre.

Milk Collection Centres

When the milk arrives at the collection centre it will be weighted and maybe a CMT test carried out. Some centres will carry out alcohol testing as an assessment of bacterial load. This test was first used around 1920. If the milk passes these tests it is poured into the bulk tank at the collection centre which is often powered by solar energy. If it fails, it goes back to the village for sale. Often, concentrates will be bought from the collection centre, put into the milk container to fed the cow at the next milking. Milk in, concentrates out!

A key advantage of dairying for small scale farmers is that it offers famers income every day. This is invaluable in countries where there is no government support or social welfare. Each person has to find their own income. This is not an easy life.

Breeding

Small scale dairy farmers have other struggles such as getting cows pregnant. There are people who can AI cows but the semen quality may be low due to storage problems or poor handling. There will be no high genomic availability semen in many areas and farmers will not understand the benefits from this.

In many cases, the cow is walked to the local bull. She might stay there until she has been served several times. The farmer then has to plan his feeding, watering and milking when the cow is away from home. There is a disease risk from using bulls and other risks from coming into contact with other cows and animals on the way. Cows are rarely checked for pregnancy; they calve when they calve. The same is true for maiden heifers.

Most commercial farmers will use AI and have much better fertility performance supported by computerised records. Many carry out pregnancy diagnosis and use heat detection aids.

Neonatal Mortality

Neonatal mortality is a problem throughout Africa for people and animals. They just accept death in the young. They don't realise the importance of colostrum management in calves and the impact that this can have on calf morbidity and mortality. Once it has been explained, this can change the performance of calf rearing but it can be difficult to get basic messages through.

Commercial Farmers

Commercial farmers tend to be either white or better educated local Africans. The herd size can be highly variable from 30 cows up to 2,000 cows. They have the same problems with infrastructure.

For example, there is one DeLaval dealer who looks after farmers in Zimbabwe and Zambia and has two people working with him. He might be away fitting a parlour for two or three weeks and so service or breakdowns become more challenging. Parts have to come via South Africa and then into whichever country he is working. These can be delayed at border posts for a month or more. Farmers carry a wide range of spare parts and cobble together repairs to keep parlours running.

They have the same problems with lack of vets, advisors, and supply of products. In fact, it's even worse than that as they are often considered to be a charity to help small scale producers and support various events and local politicians as they are perceived to be a soft touch due to their scale.

Most commercial farmers will have milking parlours, tractors and be fully mechanised. They employ large amounts of labour and one worker for every 8 to 10 cows is common. Expect three or four people in any parlour, cow pushers, a team of people feeding, heat spotters, house maids, gardeners, drivers, buyers etc. It's all about creating employment and some of the large scale farmers will be employing well over 200 people who are totally dependent on the farmer.

Commercial farmers will house their cows in corrals and some have freestall barns but these are very uncommon. Shade for cows is variable. Trees would be ideal to offer some shade from the sun in the middle of the day but many farmers cut these down to make more space. Shade over the feed areas is becoming more popular to preserve the freshness of food and maximise feed intakes.

Electricity is always a problem as is water. Farmers have to have their own generators which are very expensive to run. They will often irrigate and draw water from far away. They can afford to get some outside advice and go and visit other countries to learn and some do, others do not.

Many commercial farmers will use mixer wagons but some of these are quite elderly and in need of some TLC. The real problem with feeding cows is the quality of the raw ingredients as many poor quality products are dumped on Africa. Nutritional content can be very variable. Key ingredients run out of stock and so at times rations have to change suddenly and the rumen of the cow suffers. Ration formulation is challenging.

Commercial farmers are likely to have a range of enterprises; potatoes, crops, tobacco, fruit, beef and this helps when there are market variations. Staff tend to be better trained and they will have housing provided on the farm along with some land to grow their own crops. Some larger farms employ teachers, a doctor etc. Commercial farmers look after their staff well, but if staff steal or break the rules they are sacked on the spot and have to leave with their family.

Is There a Dairy Cow that is Perfect for the Tropics?

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Introduction

Colombia is located in the northwest corner of South America near to the equator. The climate is characterized as tropical and isothermal and is characterized by significant variation of climate between regions of the country. The diversity of climates in Colombia is a function of the variation in altitude, temperature, humidity, winds and rainfall. The variation in precipitation and temperature caused by the Intertropical Convergence Zone is another characteristic of the Colombian climate (CENICAFE, 2016). There are only two distinct seasons in Colombia: the wet season (also known as winter) and the dry season (also known as summer). In some regions the precipitation may be as high as 2500 mm/year or as low as 500 mm/year, and the temperature will vary between 25 and 35 °C, while in the other regions temperatures will range between 14 and 18 °C (CENICAFE, 2016; IDEAM, 2016).

The wide variation in climate influences the milk production in Colombia. Consequently, milk productivity is highly variable, ranging from around 15 kg/d in the most productive regions (e.g. Sabana de Bogota, north of Antioquia) to around 4 kg/d in the least productive ones (e.g. Caribbean region, Llanos Orientales). Every type of tropical dairy system is represented in a country such as Colombia. Although annual milk production in Colombia is lower compared to other countries in Latin America, it is the fourth largest milk producing country in Latin America after Brazil, Mexico and Argentina.

The difference in climate among the geographical regions is also the main reason for the many different breeds of cows used in Colombia. Usually, dairy cows in herds located in the most productive regions belong to European breeds, mostly Holstein and Normande; while cows in the least productive zones belong to local breeds and crossbreds with Zebu cattle (e.g. Brahman and Gyr). The latter are more resistant to high temperature and humidity. Therefore, milk production is varies by both breed and region. Furthermore, milk quality is also highly variable.

Recent studies have indicated that *Streptococcus agalactiae* is highly prevalent in bulk tank milk in Colombian herds, ranging from 20% to 42% (Keefe et al., 2010). Dairy herds in Sabana de Bogota, which is one of the most productive regions of Colombia, have a lower prevalence of *Strep. agalactiae* compared to Antioquia, where milk production is also high. Although the milk production system in lower regions of Colombia may be quite different compared to Sabana de Bogota, the prevalence of *Strep. agalactiae* was similar (i.e. approximately 20%). The main characteristics of these regions are described further.

The *Strep. agalactiae* prevalence in bulk tank milk in dairy herds located in the Coffee Triangle region is also high according to studies conducted at the milk quality lab of Universidad de Caldas (Velasco-Bolaños et al., 2014). The prevalence for this region was 53.3%, which was higher compared to the prevalence reported previously by Keefe et al. (2010). Dairy herds

located in lowlands of the Coffee Triangle region (below 1700 meters above sea level) had a prevalence of 42% (95% CI: 0.33; 0.52) compared to 68% (95% CI: 0.60; 0.77) in herds in highlands within the same region (above 1700 m a.s.l.) (Laboratory of Milk Quality at Universidad de Caldas, unpublished data).

Given the variation and extremes of climate experienced by cows, depending on region and altitude, the ideal cow for tropical regions will have characteristics that are typically quite different from the ideal dairy cows for temperate regions. The objective of this short talk is to discuss the characteristics of the cows typically found in dairy herds located in tropical regions, using Colombia as the example because almost every type of tropical dairy system is represented in this country. Finally, I will consider if there is a breed of cow that is ideal for supporting a sustainable dairy industry in the tropics.

Dairy Cows and Milk Production in the Tropics

Milk production has increased in the last decade in Latin America. Countries, such as Brazil and Colombia, have experienced a significant rise in milk productivity during the decade from 2000 to 2010 (FAO, 2016). However, dairy production in tropical countries faces different challenges, such as lack of standardized systems for milk production, low quality forages, high humidity, high temperature, tick-transmitted diseases, among others (Berman, 2011). Consequently, milk productivity is low (Table 1).

Table 1. Means and standard error of milk yield (kg) at the beginning of lactation and at different times of the year in four different crossbreds of Holstein-Zebu cows in Brazil (Glória et al., 2012).

Variable	Milk yield (mean ± SE)
Crossbred:	
Holstein x Gyr	8.58 ± 0.16
Holstein x Guzerat	8.01 ± 0.30
Holstein x Nellore	7.21 ± 0.33
Holstein x Zebu	9.07 ± 0.42
Season of calving:	
Onset of rainy season	7.91 ± 0.25
End of rainy season	8.90 ± 0.26
Onset of dry season	8.54 ± 0.25
End of dry season	7.51 ± 0.31

The proportion of Holstein in the crossbred composition may affect the amount of milk produced during lactation, and the duration of lactation. Brazilian studies conducted in lowland herds indicated that crossbreds with a proportion of 50% of Holstein and 50% of Zebu cattle had, on average, a 305-d corrected milk production of 2674 ± 145 kg compared to 3279 ± 152 kg when Holstein proportion increased up to 87.5% (Perotto et al., 2010). However, those proportions of Holsteins would be not applicable to all climates. An increase of 41 d in the duration of lactation when the proportion of Holstein genes in the crossbred increased from 50% to 87.5% was also found in the same study. The authors indicated that climate has a significant effect on the quantity and quality of pastures, which may affect the productivity of the cows in tropical and

sub-tropical regions. Further studies have shown also that the time of the year when cows calve may affect both the milk production over the entire lactation and the duration of lactation in crossbred cows (Berman, 2011; Glória et al., 2012).

According to the information of the Association of Gyrolando Breeders, the breed is essentially a crossbreeding between Holstein (5/8) and Gyr (3/8); however, any genetic proportion between Holstein and Gyr breeds might be considered as the starting point to get a purebred Gyrolando (GIROLANDO, 2016). The association started official milk testing back in 2005. They report an average of 6600 kg of milk per lactation (non corrected) for the trimester from June to August 2016. This is an increase of 27.3% in milk production per lactation compared to the same period in 2005 (GIROLANDO, 2016). However, Beman (2011) states that either environmental conditions or genetic potential may have imposed a ceiling to milk yield.

The most recent report of milk production per lactation in cows under official milk testing was higher in comparison to the means previously reported for crossbred Holstein x Zebu cows (Perotto et al., 2010; Glória et al., 2012).

An evaluation of the milk production per lactation in crossbred Holstein x Gyr cows in Colombia indicated that milk production per lactation might be similar to the means observed in Brazilian studies (Perotto et al., 2010; Glória et al., 2012) (Table 2), but lower compared to the average reported in cows under official milk testing (GIROLANDO, 2016).

Table 2. Mean and standard deviation of milk yield (kg) per lactation in crossbred Gyr x Holstein cows in Colombia. Means are grouped according to parity (Motta et al., 2012).

Parity	Milk yield (mean ± SD)
1	2779 ± 910
2	2908 ± 594
3	3051 ± 486
4	3326 ± 855
5	3570 ± 803

The production system with crossbred *Bos taurus* x *Bos indicus* cows is common in herds located below 1500 m a.s.l., where environmental conditions are challenging for European or North American breeds. Therefore, milk production in these lowlands is based on tropically adapted breeds crossed with *Bos taurus* breeds. This system is usually called a ‘dual purpose’ system, as cows are destined to milk production and calves are fed for meat production (RVO, 2015).

The use of local (native) breeds for crossbreeding with *Bos taurus* is another option to improve the performance of cows producing milk in challenging environments. The analysis of lactation curves of Holstein cows compared to ‘Blanco Orejinegro’ (BON) cows, a native cattle breed to Colombia, showed a higher production of Holstein cows; however, native breeds and crossbred cows are considered more adapted to tropical environments (López et al., 2001; Cañas et al., 2008; Berman, 2011). A study conducted late in 1990s showed that accumulated milk production

of crossbred cows BON x Holstein was 3351 kg in 259 d, while Holstein cows produced 6154 kg of milk in 330 d (Quijano and Montoya, 2000).

The dairy herds located in highlands (i.e. above 1500 m a.s.l.) are managed as a production system usually called a 'specialized system'. The specialized system of highlands is characterized by a more intensive use of land, which means a higher stock density compared to the use of land in dual purpose systems. These systems are usually located close to urban centers, and farmers are engaged in the exploitation of pure dairy breeds (e.g. Holstein, Normande, Ayrshire, and Jersey) or cows with a high proportion of genes from *Bos taurus* dairy breeds. Feed for this livestock is based on rotational grazing, and cows are supplemented with concentrates depending upon the level of milk yield. Cows are milked twice a day (RVO, 2015). As a consequence, milk production in highlands is higher than that found in lowlands (Table 3). Although rotational grazing may be practiced in lowlands, is not a common practice to all lowland herds.

Table 3. Milk yield (kg) adjusted to 305 d in Holstein, Normande, and Holstein x Normande cows from the dairy herd of Universidad de Caldas in Caldas province, Colombia. The herd is located at 2280 m a.s.l. Cows are on grazing all year round, supplemented with concentrates according to milk yield, and milked twice a day (Unpublished data).

Breed	Adjusted milk yield (mean ± SD)	
	Per day	Per lactation
Holstein	24.8 ± 6.4	7577 ± 1957
Normande	17.2 ± 4.8	5241 ± 1446
Holstein (50%) x Normande (50%)	26.7 ± 4.3	8152 ± 1300

According to the information above, there are substantial differences between the 'dual purpose' and 'specialized' systems (Table 4), which means that the type of cow and her characteristics for both production systems should be different.

Table 4. Summary of the characteristics of both milk production systems 'dual purpose' and 'specialized' in Colombia. Adapted from: RVO (2015).

Characteristic	Dual Purpose	Specialized
Approximate number of lactating cows (x1000)*	7,900	2,400
Number of farms	174,211	29,865
Altitude (m a.s.l.)	< 1,700	> 1,700
Main location in country	Coastal and interior	Interior
Average temperature	> 20 °C	≤ 18 °C
Quality of pastures	Low (low protein, low energy, high in detergent fibers)	Higher compared to lowlands
Grazing system	Extensive use, rotational in some herds	Rotational
Use of concentrates	Variable, usually not	According to milk yield
Predominant breeds	Crossbreds	Holstein, Normande, Jersey
Presence of calf for milking**	While this is a common practice, some herds do not follow that	Not a common practice, but it may be found in some Normande herds
Non-corrected milk yield (kg/cow/d)	4 - 6	15 - 17
Overall milk yield per year (million of kg/year)	3,639	2,980
Milk quality	High in milk solids, low average for milk hygiene	Low in milk solids, better milk hygiene

*This number might be lower according to the information of FEDEGAN, 2016 (http://www.fedegan.org.co).
**The calf is tethered to the cow for stimulating the milk letdown.

In particular, climate characteristics are differ greatly between lowlands and highlands. Cows in lowlands must be more tolerant to high temperature and high humidity, high level of exposure to ticks and some other external parasites, and need to be able to produce even if they are grazing on low quality pastures. The proportion of *Bos taurus* genes in crossbred cows located in dual purpose herds may affect their resistance to the factors above mentioned (Berman, 2011). Therefore, it is necessary to keep a balance of *Bos taurus* genes at levels that do not affect the performance of the cattle, when they are kept in lowland herds.

Warm climate breeds (e.g. *Bos indicus* and native breeds) are well adapted to challenging climate conditions, which may be exploited to improve the performance of the milk-producing cows exposed to heat stress in warm climates and mitigate the effect of warm periods in cooler climates (e.g. in highlands) (Berman, 2011). Crossing with *Bos indicus* may reduce milk productivity when *Bos taurus* dairy breeds are adapted to the climate conditions in highlands, where temperature is lower than observed in lowlands. Nevertheless, Berman (2011) indicates that milk production in warm climates will depend more upon heat stress relief (i.e. selection for heat tolerance, time to develop heat tolerance, hair coat, shadow, etc.) than upon breed type.

Crossing with *Bos indicus* genes is an alternative to improve milk production in tropical and sub-tropical regions. Some advantages may be heterosis (hybrid vigor), adaptation to heat stress, lower maintenance requirements, now accepted by NRC Subcommittee on Beef Cattle Nutrition, and a better adaptation to periods of low pasture growth.

Milk Quality

Harvesting high quality milk is a must in the tropical dairy herds. However, at least in Colombia, the milk quality (i.e. composition and hygiene) is variable depending on the production system (Table 5).

Table 5. Milk composition in Colombian dairy herds grouped by altitude. Adapted from: RVO (2015).

Characteristic	Location of the herd	
	Lowlands	Highlands
Fat (%)	3.7	3.7
Protein (%)	3.3	3.1
Bacteria count (ufc/mL)	> 1,000,000	> 400,000

One important issue in Colombian herds is the positivity to *Streptococcus agalactiae*. A former study conducted in Colombia between University of Prince Edward Island, Universidad de Caldas and COLANTA has indicated that *Strep. agalactiae* is highly prevalent in Colombian herds (Table 6). The overall prevalence of infection, weighted by region, was 42%. The plant that the milk was shipped to was a significant predictor of bulk tank prevalence.

Table 6. Regional prevalence data for bulk tanks positive to *Strep. agalactiae* in Colombian herds. The study was conducted in herds that deliver milk to COLANTA processing plants (Keefe et al., 2010).

Processing plant (Altitude*)	Bulk tanks	Positive tanks	Percentage	Confidence interval
Armenia (Lowland)	36	8	22.2%	8.4 - 36.0%
Funza (Highland)	38	5	13.2%	2.2 - 24.1%
Medellin (Low and highland)	130	51	39.2%	30.8 - 47.7%
P. Rica/P. Boyacá/Baranquilla (Lowland)	36	4	11.1%	0.7 - 21.6%
San Pedro (Highland)	138	53	38.4%	30.2 - 46.6%
Santa Rosa (Highland)	60	34	56.7%	44.0 - 69.3%
Yarumal/Frontino (Highland)	60	35	58.3%	45.7 - 70.9%

*Altitude refers to the main location of dairy herds that deliver milk to that specific processing plant.

The risk of being *Strep. agalactiae* positive was strongly associated with milking technique beyond region (Table 7). The prevalence of *Strep. agalactiae* infection in herds that reported machine milking (i.e. usually in highlands, n=144) or both machine and manual (n=17) was 14.7% and 17.0%, respectively. The prevalence of infection in hand-milked herds (usually in lowlands) was significantly higher at 54.6% ($p < 0.05$).

Table 7. Predicted somatic cell counts for bulk tank milk, based on *Strep. agalactiae* infection status and milking technique. Usually machine milking system is found in highlands, while manual milking is done in lowlands (Keefe et al., 2010)

Type of milking system	Bulk tank infection status			
	Negative		Positive	
	No. of herds	SCC	No. of herds	SCC
Machine	124	352,664	20	477,418
Manual	145	528,770	159	713,434
Both	14	478,982	3	647,572

Further studies conducted at Universidad de Caldas indicated that the Strep. agalactiae prevalence in herds of the Coffee Triangle Region was even higher than that previously reported by Keefe et al. (2010). The following 2 x 2 table shows the results found after a longitudinal study conducted between 2013 and 2015 in 230 herds of the Coffee Triangle Region in Colombia (Table 8).

Table 8. Number of herds of Coffee Triangle Region of Colombia that were negative and positive to *Strep. agalactiae* in a longitudinal study between 2013 and 2015. Herd was considered positive if, at least, one bulk tank milk sample was positive. Twelve milk samples were fortnightly collected in each herd (Ceballos et al., 2016. Universidad de Caldas, unpublished results).

Altitude	*Streptococcus agalactiae* in BT milk		Total
	Negative	Positive	
Lowland herds (< 1700 m a.s.l.)	40	86	126
Highland herds (> 1700 m a.s.l.)	60	44	104
Total	100	130	230

Other mastitis-causing pathogens are found in tropical herds, such as *Staph. aureus*, *Strep. uberis*, and non-aureus staphylococci. However, Gram negatives and coliforms are less common than found in temperate countries.

The main challenges that the dairy industry faces in tropical countries are the lack of standardized milking procedures and treatment protocols, and the lack of regular milk testing as an aid to udder health programs. These factors might be associated with a high prevalence of mastitis compared to temperate regions. Nevertheless, more research needs to be done to tell if the variation in milk quality might be a result of breed, climatic conditions (e.g. temperature and humidity), milking systems (e.g. presence of calf or not), milking hygiene management (e.g. following strict milk procedures), nutrition or other factors, or some combination among these factors.

Conclusions

The heat stress, external parasites and some other tropical diseases are issues to consider when looking for an "ideal" type of cow to produce milk in the tropical and sub-tropical regions of the world. This means that crossing adapted cows with foreign breeds might be an alternative to improve the resistance of the animal to challenging conditions.

On the other hand, the prevalence of *Strep. agalactiae* infected herds not only in Colombia but in other tropical countries is high. More research is needed to understand how breed and overall management conditions in lowland herds affect milk quality.

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References

Berman, A. 2011. Invited review: Are adaptations present to support dairy cattle productivity in warm climates? J. Dairy Sci. 94:2147-2158.

Cañas, J.J., L.F. Restrep, J. Ochoa, A. Echeverri, and M. Cerón-Muñoz. 2008. Estimación de las curvas de lactancia en ganado Holstein y BON x Holstein en trópico alto colombiano (Spanish). Rev. Lasallista Invest. 6:35-42.

CENICAFE. 2016. Anuario Meteorologico Cafetero 2015 (Spanish). CENICAFE, Chinchiná, Colombia.

FAO. 2016. FAOSTAT: Total Milk Production. Nov. 22, 2016. http://faostat3.fao.org/browse/Q/QL/E.

GIROLANDO. 2016. Associação Girolando (Portuguese). Nov. 22, 2016. http://www.girolando.com.br.

Glória, J.R.d., J.A. Garcia Bergmann, C.R. Quirino, J.R. Mendes Ruas, J.C. Campos Pereira, R. Brag Reis, S. Gesteira Coelho, and M. de Almeida e Silva. 2012. Environmental and genetic effects on the lactation curves of four genetic groups of crossbred Holstein-Zebu cows. R. Bras. Zootec. 41:2309-2315.

IDEAM. 2016. Instituto de Hidrología, Meteorología y Estudios Ambientales (Spanish). Nov. 18, 2016. http://www.ideam.gov.co.

Keefe, G.P., M. Chaffer, A. Ceballos, M. Londoño, M. Toro, and M.I. Montoya. 2010. Prevalence of *Streptococcus agalactiae* in cooling tanks of COLANTA. In: Proc. VII Seminario Internacional de Competitividad en Carne y Leche. COLANTA, Medellin, Colombia.

López, A., O. Saldarriaga, A.E. Arango, M.T. Rugeles, F.N. Zuluaga, M. Olivera, N. Bermúdez, G. Bedoya, and J.E. Ossa. 2001. Ganado Blanco Orejinegro (BON): Una alternativa para la producción en Colombia (Spanish). Rev. Col. Cs. Pec. 14:121-128.

Motta, P.A., L.G. Rivera, A. Mariño, and C.E. Lizcano. 2012. Desempeño productivo y reproductivo de vacas F1 Gyr x Holstein en clima cálido colombiano (Spanish). Rev. Col. Cs. Pec. 6:17-23.

Perotto, D., I.A. Kroetz, and J.L. da Rocha. 2010. Milk production of crossbred Holstein x Zebu cows in the northeastern region of Paraná State. R. Bras. Zootec. 39:758-764.

Quijano, J.H., and C. Montoya. 2000. Comparación productiva de vacas Holstein y F1 Blanco Orejinero (BON) x Holstein 1. Producción y calidad de la leche (Spanish). Rev. Fac. Nal. Agr. Medellin 53:1115-1128.

RVO. 2015. Mooooi dairy opportunities for Colombia-Dutch collaboration. The Hague, Netherlands.

Velasco-Bolaños, J., C. Cobo, P.C. Duque, N.A. Villa, L. Lasso, and A. Ceballos. 2014. Prevalencia y factores de riesgo para *Streptococcus agalactiae* en tanques de leche en el departamento de Caldas, Colombia (Spanish). In: Proc. Annual Meeting of RELIM. RELIM, San Carlos Costa Rica.

Is There an Ideal Automatic Milking System Cow and how is She Different from an Ideal Parlor-milked Cow?

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Introduction

The first automatic milking system (AMS) installations on commercial farms were established in the Netherlands in 1992. The majority of the robotic milking stations are still located in Western and Northern Europe, but numbers are now rapidly growing in North America and other countries over the globe. An estimated 38 000 robotic milking stations now operate worldwide and in North America, the U.S. has more than 1200 and Canada around 1800. In order to exploit the full potential of an AMS operation it is imperative to follow through with daily and weekly routines, but what about the cow? Is there an ideal AMS cow and is she different from an ideal parlor-milked cow? What is current knowledge on genetic evaluations of milkability, mastitis and temperament in AMS's? How is udder health and milkability associated? Which traits are the breeding companies taking into account when offering so called "robot bulls"? How confident can we be about the traits claimed to create an "ideal" AMS cow? This paper gives an overview on current knowledge on the "ideal" phenotypic traits for a cow in an AMS environment.

Is There Room for Improvement?

Profitable Robotic milking is about 24/7 milk flow

The milking station is built around one milking point and the milking equipment could therefore, from a design point of view, be said to resemble an advanced version of bucket milking e.g. one single milking point serving 50 – 70 cows around the clock. To ensure good economy in any milking installation, and maybe particularly so with an AMS, the milking box needs to be efficiently utilized so that a maximum amount of energy corrected milk is produced per day (Byskov et al., 2013). For AMS, a key figure that has been mentioned to aim for is >2000 liters of milk per day and robot (Carlström, 2014; Rodenburg, 2016). Milk flow rate, expressed as average flow rate (AFR) (Carlström et al., 2014), has together with the number of cows per station, been shown to be the most important variable influencing the daily milk yield per AMS (Castro et al., 2012). The potential for genetic improvement of flow rates is definitely there, because very high variation within breed has been shown both for conventional milking systems (CMS) as well as for AMS (Zwald et al., 2005; Byskov et al., 2013; Carlström et al., 2014).

Behavior important

One difference between AMS and CMS is that the former has demands on cows to express a higher degree of activity and to visit the milking station several times a day on their own initiative. This is particularly important in early lactation, because more milkings increases the possibilities to reach a high lactation yield. Thus, it has been proposed to include aspects of behavior and temperament into breeding goals (König et al., 2006). Behavioral traits may be particularly important for first calvers as it has been found that they might be slow adapters in terms of daily visits (Vosman et al., 2014; Bossen, 2014). However, fresh first calvers are not the

only ones that need to be active, and this feature is valid for all cows in the AMS herd. It is most likely that activity is associated with both behavioral variation and health disturbances, where particularly leg and hoof health has been pointed out as an important feature to achieve targeted AMS performance (Bach et al., 2007; Borderas et al., 2008).

All these data – can they be used?
Automatic milking systems are equipped with sensors, which monitor the milking process as well as the functioning of the system, and the sensor records are automatically stored in databases (de Koning, 2010). Hence, AMS provide an opportunity to capture repeated in-line measured observations such as milkability. AMS data allows us to expand the milkability concept to include not only the traditional milking speed traits, flow rate and milking duration, but also, for example, traits concerning the occupation time in the milking box, teat cup attachment failures as well as milking interval, number of visits, udder and teat conformation traits etc. It is clear that this and other information, such as conductivity or in-line cell counting, provide highly valuable information to support management. The question remains, however, if 1) they can be used for breeding evaluation and 2) there are main differences as compared to CMS?

Breeding for Milkability
To be able to improve traits by breeding a prerequisite is to have good measurements of the trait that you want to select for and that the genetic variability is large enough. From the studies done so far it can be concluded that there are *very good opportunities to improve milkability* as the variation in the trait is large (Carlström 2014). Heritability estimates from studies looking at subjectively scored milkability as well as electronically measured flow rates are in the range of 0.1 to 0.2 (Meyer and Burnside, 1987; Lawstuen, et al., 1988; Sewalem, et al., 2011). Lately, heritabilities between 0.27-0.38 and 0.37-0.48 have been found for electronically recorded average flow rates in CMS and AMS, respectively. Similar heritability levels have been reported for the Norwegian Red (Heringstad and Bugten). Also, the trait appears to be basically the same in the two systems (Table 1), with genetic correlations of 0.97 and 0.98 for the two breeds Swedish Holstein (SH) and Swedish Red (SR), respectively (Carlström et al., 2014).

Table 1. Genetic correlations between automatic (AMS) and conventional (CMS) milking system traits including all lactations (SE in parentheses)

Trait in AMS	Trait in CMS	SH	SR
AFR	AFR	0.97 (0.04)	0.98 (0.03)
AFR	MT	-0.96 (0.04)	-0.99 (0.03)
MT	AFR	-1.00 (0.03)	-0.95 (0.04)
MT	MT	0.98 (0.04)	1.00 (0.04)
BT	AFR	-0.98 (0.04)	-0.94 (0.04)
BT	MT	0.99 (0.03)	0.93 (0.05)

AFR = average flow rate; MT = milking time; BT = box time.
SH = Swedish Holstein; SR = Swedish Red. (Carlström, et al., 2014)

Hence, there are very good opportunities to select cows for milkability based on automatic records in modern milking systems, as concluded by Byskov et al. (2013) and Carlström et al.

(2014). Also, coefficients of variation have shown to be similar across parities, ranging similarly from 27 to 31% for SH and SR. For both breeds, Average flow rate and Peak flow rate were concluded to be the same trait.

What about box time?
As the milking visits are accompanied by handling time, e.g. pre- and post-treatment plus the entrance and exit time, box time (BT) and milking time (MT) has been proposed as traits to be used for genetic evaluation purposes (Carlström et al., 2014). As a consequence, milkability data from AMS and CMS in commercial herds with SH and SR cows have been used to evaluate AFR, MT and BT. In these studies, moderate to high heritabilities and generally high repeatabilities in both breeds were found, with genetic correlations between 0.93 and 1.00 for MT and AFR in CMS compared with MT, AFR or BT in AMS (Table 1). Because the different milkability traits were highly genetically correlated, it is sufficient to include only one of the traits, either AFR or MT or BT, in the genetic evaluation.

High milk flow – a risk for udder health
Breeding for high flow rates has some risks associated to it. Several early studies have shown unfavorable relationships between milking speed and udder health (Luttinen & Juga, 1997; Boettcher et al., 1998; Zwald et al., 2005). Bulk milk somatic cell counts are indeed, with large variations, somewhat higher in AMS as compared to CMS farms. It is unlikely this being due to AMS farmers selecting cows with high flow rates as several other risk factors with no causal relationship to milking speed have been indicated (Hallén Sandgren, 2015). It is known that there is an optimum flow rate in relation to udder health, which has been found for both SH and SR in AMS, although it is lower than the current averages for the two breeds (Carlström, 2014). Genetic correlations between milking speed and clinical mastitis has not shown to be consistent between SH and SR. For SH results of clinical mastitis are in agreement with the SCC results and in the range 0.32-0.48, and an increased risk of clinical cases associated with fast-milking cows. In the future, emphasis should be put on including udder health and milkability together in a total merit index (TMI).

Breeding for Udder Conformation
Udder conformation and milking performance
High correlations between udder and teat conformation from in-line measured teat coordinates and corresponding traits scored by classifiers clearly demonstrate the potential for the use of information from teat coordinates, see Table 2 (Byskov et al., 2013). Shallow udders with short and thin teats are genetically associated with higher milking speed (Carlström, 2014). More detailed Swedish studies on in-line measured traits by Carlström (2014) have shown that handling time was associated with udder depth, udder balance, front teat placement, teat length and thickness for SR. The results show that too much emphasis on milking speed could lead to cows with too short teats that eventually could become a problem for teat cup attachment. The importance of rear teat placement to get a proper teat cup attachment is another trait that has become more in focus, and particularly so in the HF breed when breeding cows for AMS purposes.

Udder conformation and udder health
The genetic correlations between udder conformation traits and subclinical and clinical mastitis, generally of the order 0.2-0.4, shows that shallow udders and strong fore udder attachments are the most important traits for good udder health (Carlström, 2014). Thin teats in SH and close front teat placement in SR have also been related to good udder health. These results are generally in agreement with previous studies (Boettcher et al., 1998; Rupp and Boichard, 1999; Dube et al., 2009).

Table 2. Heritabilities and genetic correlations between udder traits measured in AMS and scored by classifiers. (SE in parentheses)

Trait	h^2 AMS	h^2 Assessments	r_g
Front teat placement	0.46 (0.06)	0.31 (0.01)	0.92 (0.04)
Rear teat placement	0.38 (0.05)	0.32 (0.01)	0.94 (0.04)
Distance rear and front teats	0.46 (0.09)	-	-
Udder balance	0.44 (0.07)	0.22 (0.01)	0.90 (0.04)
Udder depth	0.65 (0.06)	0.42 (0.01)	0.94 (0.02)

(Byskov et al., 2013)

Additional Traits – Behavior
Previously, on a global scale, almost all genetic selection in dairy cow breeds has been based on milk yield and exterior conformation. Lately it has been more generally understood, also outside the Scandinavian countries, that targeting also the low and medium heritability traits for health and fertility is likely to be much more successful in order to reach a total genetic merit related to profitability. Various traits related to cow longevity or lifetime productivity has thus been introduced and, as an example, the cow livability index (C.LIV) was recently released by the Council on Dairy Cattle Breeding in the US. However, even though the opportunity to breed for behavior has been known since long (Dickson et al., 1970; Baehr, 1983), it has been very rarely exploited. As the cow in the AMS is expected to exert all activities in the barn "all by herself" it is time to understand and consider how to select for an "appropriate", maybe more flexible, behavior such as number of visits to the station, competiveness for resources etc.

Some traits related to behavior, such as handling and box time, habituation to the robot, milking intervals, incomplete milkings and attachment failures have been studied (Konig et al., 2006, Carlström et al., 2013; Vosman et al., 2014). Particularly for the attachment failures and temperament the heritability seem to be high enough for both SH and SR breeds (0.21-0.31), and the genetic correlation between the traits is positive (0.44-0.71), so both can be targeted for genetic improvement in parallel. Handling time has a somewhat lower heritability (0.05-0.15) but is as described to be highly correlated to AFR and hence already taken into account in that trait. Regarding milking frequency, heritabilities of 0.16 and 0.22 with genetic correlations of 0.46 to 0.57 with test-day milk yield have been reported (Konig et al., 2006), which is higher than the values reported by others, i.e. 0.11 (Vosman, 2015). Breeding for shorter milking intervals is thus feasible. Would breeding for improved claw health have the same effect? As said, that would require solid phenotypic data, but would be very beneficial in any milking system. One of the challenges with AMS is, as mentioned, to achieve a high enough milking frequency in fresh first calvers. CRV, the Dutch breeding company, has developed an index

evaluating the habituation of heifers to the system, but so far no independent research has validated the efficiency of the index and the heritability is rather low (0.07).

How are the Breeding Companies Acting?

CRV has introduced some specific traits for breeding of Holstein cows in AMS. The traits they are presenting are AMS efficiency (EF) which is the produced amount of milk in kg per total box time in minutes, milking interval (MI) which is the time between 2 consecutive successful milkings in minutes. They have also developed, but not released, an index for habituation of heifers (HH), reflecting the time period a heifer needs to get familiar with the AMS. The heritabilities for different lactations are reported to be 0.17 – 0.19 for EF, 0.06 – 0.08, for MI, and 0.07 for HH (Vosman, 2015), (Table 3). Genetic correlations between EF and Milk flow is, as expected, above 0.9 and the correlation between same traits measured in heifers and multiparous cows are moderate to high. The traits are not included in the Net merit index (Vosman et al., 2014). No additional emphasis is put on udder conformation. Another breeding company from the Netherlands, Altagenetics, put emphasis on rear teat placement, teat length, udder depth, rear leg rear view and milking speed.

Table 3. CRV AMS traits: Heritability (h^2), repeatability and genetic variance

Trait	h^2	Repeatability	Genetic variance
AMS efficiency L1[1]	0.19	0.58	0.2 kg /min
Milking interval L1	0.08	0.34	40.4 min
Habituation of heifers	0.07		20.3 min
AMS efficiency L +2[2]	0.17	0.56	0.22 kg/min
Milking interval L +2	0.06	0.32	-35.5 min

(Vosman, J., personal communication Nov 2016) [1] 1st Lactation, [2] ≥2nd Lactation

Some of the companies put more emphasis on udder health, when offering bulls with genetics for high milk flows. Thus, DHV in Germany, offering the RZRobot bulls, increase the weight on udder health combined with minimum levels for milk flow, rear teat placement and teat length. The Nordic breeding company, Viking Genetics, has chosen a somewhat different approach. Their general view is that the best cows are the same in parlors as in AMS (personal communication, L. Nielsen). Hence, keeping the ingoing parameters in the net total merit (NTM) index the same in their Robot index (RoboVik) but rearranging the weights on some traits directed towards breeding for AMS (Table 4). The ones they are tuning up are milk flow, udder balance and rear teat placement, based on the research by Carlström (2014) but also for udder health (introducing a minimum level), claw health and legs.

Table 4. Viking Genetics % Weights in NTM and RoboVik

Trait	% Weight NTM	% Weight Robovik
Udder health	10.8	14.3
Claw health	1.8	4.5
Legs	2.9	3.2
Udder conformation (several traits)		Increased

(Stålhammar, H., personal communication. Nov. 2016)

Conclusions

- The best cow in an AMS will be a top cow also in CMS but some traits increase in importance.
- Udder conformation is a trait that is clearly more important in AMS than in other milking systems and particularly in relation to attachment failures and kick offs.
- Average flow rate / Box time / AMS efficiency are highly correlated and flow rate is even more important for profitability than in CMS.
- Breeding for very high flows, box time or AMS efficiency should be avoided if not taking very reliable and solid breeding values and goals for mastitis into account!
- Breeding for shorter milking intervals is feasible. But is it economically justified?
- Heifer habituation index has a low heritability and improved management, such as handling and smooth introduction of heifers, is probably a better remedy.
- Data from the AMS and its sensors can be used for genetic evaluation. However, it should be validated between milking machine brands and evaluated in relation to data from CMS.
- There is a need for standardization and validation of the breeding indexes that are offered by different companies.
- The modern dairy cow has, not only, to fit into modern milking systems but also to stay healthy and vigorous throughout her entire lifespan. Breeding for health traits and longevity is a necessity for profitability and cow welfare.

References

Bach, A., M. Dinare´s, M. Devant, and X. Carre. 2007. Associations between lameness and production, feeding and milking attendance of Holstein cows milked with an automatic milking system. J. Dairy Res. 74:40-46.

Baehr, J. 1983. Verhalten von Milchkühen in Laufsta¨ llen. PhD Dissertation, Christian-Albrecht University, Kiel, Germany.

Boettcher, P.J., J.C.M. Dekkers, and B.W. Kolstad. 1998. Development of an udder health index for sire selection based on somatic cell score, udder conformation, and milking speed. J. Dairy Sci. 81:1157-1168.

Borderas, T.F., A. Fournier, J. Rushen, and A.M.B. de Passille. 2008. Effect of lameness on dairy cows visits to automatic milking systems. Can. J. Anim. Sc. 88:1-8.

Bossen, D. 2014 AMS – Feeding and management, Knowledge Centre for Agriculture, Denmark. Internal DeLaval seminar 2014-05-21.

Byskov, K., L.H. Buch, and G.P Aamand. 2012. Possibilities of implementing measures from automatic milking systems in routine evaluations of udder conformation and milking speed. Interbull Bull. 46:28-32.

Carlström, C. 2014. Genetic Variation of In-line Recorded Milkability Traits and Associations with Udder Conformation and Health in Swedish Dairy Cattle PhD Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Carlström, C., G. Pettersson, K. Johansson, E. Strandberg, H. Stålhammar, and J. Philipsson. 2013. Feasibility of using automatic milking system data from commercial herds for genetic analysis of milkability. J. Dairy Sci. 96:5324-5332.

Carlström. C., E. Strandberg, K. Johansson, G. Pettersson, H. Stålhammar, and J. Philipsson. 2014 Genetic evaluation of in-line recorded milkability from milking parlors and automatic milking systems. J. Dairy Sci. 97:497–506.

Castro, A., J.M. Pereira C. Amiama, and J. Bueno. 2012. Estimating efficiency in automatic milking systems. J. Dairy Sci. 95:929-936.

de Koning, C.J.A.M. 2010. Automatic Milking - Common Practice on Dairy Farms, Proc. of the First North American Conference on Precision Dairy Management, pp 52–67.

Dickson, D.P., G.R. Barr, L.P. Johnson, and D.A. Wieckert. 1970. Social dominance and temperament of Holstein cows. J. Dairy Sci. 75:904–907.

Gäde, S., E. Stamer, J. Bennewitz, W. Junge, and E. Kalm. 2007. Genetic parameters for serial, automatically recorded milkability and its relationship to udder health in dairy cattle. Animal 1:787-96.

HallénSandgren, C. 2015. Maintenance of and Trouble Shooting on Milk Quality in Automatic Milking Systems, Proc. 54th Annual Meeting of the National Mastitis Council, Feb 1 – 3, Memphis, US. pp 26-36.

Heringstad, B., and H. Kjören Bugten. 2014. Genetic evaluations based on data from automatic milking systems. 39th ICAR Session, May 19-23 Berlin, Germany.

Konig, S., F. Kohn, K. Kuwan, H. Simianer, and M. Gauly. 2006. Use of repeated measures 605 analysis for evaluation of genetic background of dairy cattle behaviour in automatic milking 606 systems. J. Dairy Sci. 89:3636-3644.

Lawstuen, D.D., L.B. Hansen, G.R. Steuernagel, and L.P. Johnson. 1988. Management traits scored by dairy producers. J. Dairy Sci. 71:788-799.

Luttinen, A., and J. Juga. 1997. Genetic relationship between milk yield, somatic cell count, mastitis, milkability and leakage in Finnish dairy cattle. Interbull Bull. 15:78-83.

Meyer, K., and E.B. Burnside. 1987. Scope for a subjective assessment of milking speed. J. Dairy Sci. 70:1061-1068.

Rodenburg, J., N. Lyons, and K. Kerrisk. Changes in management strategies with adoption of robotic milking Joint Annual Meeting 2016, July 19 - 23, Salt Lake City, USA.

Rupp, R., and D. Boichard. 1999. Genetic parameters for clinical mastitis, somatic cell score, production, udder type traits, and milking ease in first lactation Holsteins. J. Dairy Sci. 82: 2198-2204.

Sewalem, A., F. Miglior, and G.J. Kistemaker. 2011. Short communication: Genetic parameters of milking temperament and milking speed in Canadian Holsteins. J. Dairy Sci. 94:512–516.

Vosman, J.J. 2015. Genetic evaluation for automatic milking system traits in the Netherlands. EEAP, Sept. 2, 2015.

Vosman, J.J., G. de Jong, and H. Eding. 2014. Breeding of cows suitable for an automatic milking system. Interbull Bull. 48:32-35.

Zwald, N.R., K.A. Weigel, Y.M. Chang, R.D. Welper, and J.S. Clay. 2005. Genetic evaluation of dairy sires for milking duration using electronically recorded milking times or their daughters. J. Dairy Sci. 88: 1192-1198.

Preventing Mastitis through Proper Management and Monitoring of The Milking System

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Introduction

As herds aspire to achieve higher levels of milk production and milk quality there is a need for more focused management of all areas of the farm. The management of the milking center is no exception to this. In order to achieve success with the milking center the first step is to document what the current baseline is for all the major areas affecting the milking center. During the analysis of this baseline data one important distinction to make is whether the opportunity areas that are identified relate to the system as a whole or to the performance of individual personnel in the system. For opportunity areas that relate to the system as a whole, changes are then implemented to optimize the performance of the milking center. These changes may relate to people and what they are doing, equipment such as unit alignment devices or liners, equipment settings such as vacuum or automatic take-offs, products such as dips or chemicals, or numerous other factors. The system as a whole is then re-evaluated and any additional changes are made to capitalize on all the benefits of the initial change. During this time an intense monitoring program is also put in place that gives management the essential feedback that is necessary to monitor the system as a whole and to manage individual personnel in the system. Coupling incremental changes with an ongoing monitoring program can help dairies consistently achieve high milk production, excellent cow health, parlor efficiency, and top quality milk.

Baseline Assessment of the System as a Whole

There are many changes that occur within a milking center over the course of days to months. Many of these changes have impacts on other aspects of the milking center beyond the individual component that was altered. This can lead to a situation that is out of control but no one recognizes this fact. In this state it will be hard for a dairy to achieve or maintain its goals of high milk production, excellent cow health, parlor efficiency, and top quality milk.

The first step is to document in detail where the herd is currently at. This serves as a baseline that can then be analyzed to determine a priority list of the changes that are needed. At QMPS this would involve a herd visit of a complete milking shift to evaluate the following parameters: milkline vacuum stability, average claw vacuum at peak flow, pulsation, unit alignment, milking routine timing, milking efficiency and throughput timing, milk flow rate analysis, teat scoring (short and long term effects), strip yields, teat end cleanliness, udder hygiene, and an assessment of the cow housing facilities. If a complete test of the milking system as recommended by the National Mastitis Council (NMC) to evaluate the vacuum levels and airflow in the milking system has not been performed in the last 6 months then this would be completed as well.

Milkline vacuum stability is assessed per NMC guidelines over an approximately 30-minute period at the milk inlet closest to the receiver jar during normal milking operations. The average claw vacuum at peak flow and the pulsation parameters are measured with the unit on the cow

and milk flowing through the claw per the NMC guidelines for dynamic testing. Unit alignment is measured using a two category scoring system (proper or improper unit alignment). This is assessed within the first two minutes after unit attachment and any three-quarter cow is not scored.

Milking routine timing focuses primarily on initial stimulation time, pre-dip contact time, and the lag time from stimulation to unit attachment. The milk flow rates of individual cows are measured using a Lactocorder® device. If the milking center has milk meters that are providing individual cow flow data this is analyzed as well.

Accurate milking efficiency and throughput timing can be a challenge to obtain at the same time as multiple new people are in the parlor performing the other measurements as this may disrupt some aspects of the routine. We have found that it may be a more accurate assessment to perform these timings during the previous shift or stay until the next shift has started. This data is recorded on a standardized form and minimally includes: time at entrance gate open, time at start of prep on first cow, time at unit attachment of first cow, time at last unit attachment, time at third to last unit detachment, time at second to last unit detachment, time at last unit detachment, and time at exit gate opened.

Teat scoring is performed within one minute of unit detachment using the Teat Club International scoring system (Mein et al., 2001). At least 20% of the herd is scored in the categories measuring the short and long term effects on the teats. As per the Teat Club International recommendations the data is summarized by cow (Reinemann et al., 2001).

Strip yields are performed immediately after unit detachment. Each teat is stripped for a maximum of 15 seconds and the total volume of milk from all 4 teats recorded. It is also noted if the milk was evenly divided between all four teats or if only one teat accounted for the majority of milk.

Teat end cleanliness is performed after teat preparation but prior to unit attachment. A four by four gauze soaked in alcohol is used to swab the teat end. The scores are recorded using a one to four category system (WestfaliaSurge, ©2005) with one being a clean teat end with no dip, dirt, or manure present and four being a teat end with large amounts of dirt or manure present. Udder cleanliness is scored using a one to four category system (Pamela Ruegg, ©2002) with one being an udder that is free of dirt or manure and four being an udder that has greater than 30% of the surface area covered with caked on dirt or manure.

Cow housing facilities, both lactating and dry, are assessed on a pen by pen basis with notes recorded on stall cleanliness, bedding amounts, and cow positioning in the stalls. The holding area and alleyways are assessed for areas of manure accumulation that could lead to splash onto teats and udders.

Distinguishing between the System as a Whole versus Individual Personnel

In analyzing the data from the initial baseline assessment and subsequent follow-up visits it is important to distinguish between those opportunity areas that relate to the system as a whole versus the performance of individual personnel within the system. This helps give the farm a clear direction for how to move forward to implement a solution to the problem.

Optimization of the System as a Whole

For those opportunity areas where the system as a whole is involved it is critical that as changes are made there needs to be follow-up that documents any changes to the related parameters. Too often we see dairies make changes but then do not re-evaluate the system as a whole and so miss the benefit of the change. By following up on changes and re-assessing the complete system you can move closer to optimizing the system for your cows. Cows do adjust over time and it is our belief that you should be continually re-evaluating your milking center to determine the next change that can be made to benefit both the business and the cows.

Management and Monitoring of Details with Individual Personnel and Equipment

The goal for any herd should be to prevent problems rather than react to existing problems. For example once a cow has mastitis the major battle has already been lost. It is true that some cows self-cure and others we can successfully treat but the milk loss has already happened.

In order to prevent problems we need to have a robust monitoring system in place that includes all aspects of the milking center. Depending on the dairy this may involve multiple managers carrying out different aspects of this monitoring system or only a single parlor manager who is responsible for all aspects. The monitoring plan needs to be written out in detail and include what specific parameters will be monitored on what frequency and by whom. It also needs to have the standards that you are monitoring against clearly written out so that it is obvious to everyone when a standard is not being met.

We need to manage the milking center in such a controlled way that the variation day to day is minimized to the greatest extent possible. The best farms that we have the luxury of evaluating are the ones that strive for the highest level of consistency. All milkers bring cows to the parlor in the exact same way on all shifts. All milkers practice excellent animal handling and perform the exact same routine in the parlor. The settings of the milking equipment are optimized for the dairy and the cows that are being milked. There is a constant monitoring plan of the milkers, equipment, and the cows to identify problems before they become catastrophes. Management knows exactly what the settings are and what is happening in the milking center at all times. This includes the cleaning of the milking system as well as it has an impact on the life of the perishable products in the milking system.

Critical Areas to Monitor and Manage on a Herd Specifically Relating to the Milking System

Scheduled monitoring and maintenance of the milking equipment by trained technicians

Scheduled monitoring and maintenance of the milking equipment is a must for all dairies but especially those that are running around the clock. There is a predictable life of individual products and these products need to be rebuilt or replaced prior to this so that problems are prevented. Too often farms have good intentions of their employees performing these routine tasks but there is not actual time set aside to do this and so they are forgotten. For the majority of dairies, they probably are farther ahead by outsourcing this work and then holding that party accountable for performing the agreed upon tasks. This also brings in an outside set of eyes that if given the appropriate conduit for information flow, can aid the dairy in noticing additional areas for improvement. If problems are consistently identified with the monitoring of milking equipment such as when pulsators are graphed, then the interval between graphing/rebuilding events needs to be shortened. This will lead to prevention or an earlier detection of the problem

and therefore reduce the risk of negative effects on cows. Routine assessment of system cleaning is also an essential part of this process as an issue with system cleaning can lead to inappropriate use of chemicals which affects the lifespan of other parts of the milking system.

Brief assessment prior to the start of every milking shift
These are the tasks that a lead milker should be responsible for performing at the start of every milking shift to help limit the number of cows put at risk due to improperly performing milking equipment.

Vacuum level on the farm gauge should be checked and recorded in a daily log. All units should be turned on and a finger inserted into each liner to make sure that at least the liner is collapsing with each pulsation cycle. All liners should be checked to make sure they are not twisted in the shell. All vents in the claws and/or liners should be opened using an appropriately sized tool. Observe all hoses, gaskets, etc. for any obvious tears. Listen for any air leaks in the entire milking center.

Convincing the farms that you work with to implement these simple checks at the start of every milking would go a long way to preventing mastitis associated with poorly functioning milking equipment based on the problems we detect on farms on a weekly basis. On a recent herd assessment of a double 18 parlor we found over 33% of the vents in the claws plugged in a herd that was not using vented liners. Milkers were not aware that the vents were plugged and based on visual assessment it appeared that some had been plugged for a while. On some of the cows milked with a claw that had a plugged vent, the average claw vacuum at peak flow was reaching over 14.5”Hg (49.1 kPa) versus the normal level for this herd of 12.3”Hg (41.7 kPa). A conservative estimate for a single milking was that we put over 300 cows at an increased risk for mastitis based solely on an easily preventable problem.

Communication between milkers, managers, and owners about improperly functioning equipment
It is worth the effort to help all parties (milkers, managers, and owners) come to a clear understanding and consensus about the level of urgency of correcting issues that arise with various components of the milking system. In the dairies that we evaluate there is wide variation in the lag time between farms noticing and correcting an issue such as a pulsator that is non-functional on one side or an automatic take-off that is not working correctly. Simply educating milkers on what they should be looking for and defining who they should notify is a good first step. Then at the management level there needs to be a clear outline of who is responsible for making sure that the improperly functioning equipment is fixed and a definition of the expected time frame to complete the task. On some dairies, this has been successfully implemented with a simple checklist that is signed off on each day by the manager responsible. On other dairies with parlor automation, reviewing the error reports on the daily print outs can also facilitate this task and should be encouraged. Reducing the amount of time to correct an issue with improper functioning equipment such as a pulsator or an automatic take-off that is continually put on manually can help to reduce the risk of mastitis on the dairies that you work with.

Unit Alignment

Unit alignment is another area to monitor and manage to achieve top performance from your cows. Unit alignment can have a significant influence on the milking speed of an individual quarter and can therefore influence unit on time and potentially the time spent in low flow of the other three quarters which milk out at normal speed. This increases the risk for teat damage and liner slips on these three teats that spend a considerable time in low flow. Additionally, many cows will experience discomfort in this situation and start to kick at the unit which potentially leads to additional liner slips, forced unit removal, and increased contamination with dirt and manure of the claw and shells. Poor unit alignment can also significantly influence parlor efficiency by increasing unit on time and increasing the number of reattached units.

Unit alignment can be a system problem with improper design of the parlor or of the unit alignment devices. It can also be a maintenance issue with the unit alignment devices in poor repair or non-functional. This needs to be evaluated on an individual parlor by parlor basis as there is no one system fits all. Any reasonable options to promote better unit alignment of the whole system should be explored.

Unit alignment can also be an individual milker issue and therefore needs to be part of a monitoring program for milkers. If milkers are not properly aligning units they need to be re-trained and re-assessed. The small amount of time that milkers need to spend properly aligning units can pay large dividends in increasing parlor efficiency and reducing the risk for mastitis.

Conclusion

In order to achieve excellent milk production, have healthy cows, maintain parlor efficiency, and produce high quality milk a dairy has to continually focus on the details of how the milking center is managed. This starts with a baseline assessment and then moves to the optimization of the milking center for the dairy by making incremental changes with a re-assessment after each one. Along the way a strict monitoring program is implemented so that there is very little room for undetected procedural drift in the system. Following this regimented program will help herds not only move to the next level but consistently stay at this higher level.

References

Mein, G.A., F. Neijenhuis, W.F. Morgan, D.J. Reinemann, J.E. Hillerton, J.R. Baines, I. Ohnstad, M.D. Rasmussen, L. Timms, J.S. Britt, R. Farnsworth, N. Cook, and T. Hemling. 2001. Evaluation of Bovine Teat Condition in Commercial Dairy Herds: 1. Non-Infectious Factors. AABP-NMC International Symposium on Mastitis and Milk Quality.

Reinemann, D.J., M.D. Rasmussen, S. LeMire, F. Neijenhuis, G.A. Mein, J.F. Hillerton, W.F. Morgan, L. Timms, N. Cook, R. Farnsworth, J.R. Baines, and T. Hemling. 2001. Evaluation of Bovine Teat Condition in Commercial Dairy Herds: 3. Getting the Numbers Right. AABP-NMC International Symposium on Mastitis and Milk Quality.

Ruegg, P. Copyright 2002. Udder Hygiene Scoring Chart: Available at: http://milkquality.wisc.edu/wp-content/uploads/2011/09/udder-hygiene-scoring-chart.pdf.

WestfaliaSurge. Copyright 2005. Teat Cleanliness Scoring Sheet. Available at: www.westfalia.com/Images/Scorecard%20Handout_tcm93-19407.pdf.

Optimizing Udder Health through Facility Design

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Introduction

While the U.S. dairy industry has seen improvements in herd bulk tank somatic cell counts over recent years, clinical mastitis largely due to environmental pathogens, remains a significant hurdle yet to be resolved in many expanding, confinement freestall housed facilities. There are no industry benchmarks for clinical mastitis rate available in the United States, but we visit "well-managed" high milk producing herds, where the cow case rate of clinical mastitis exceeds 100 cases per 100 cows per year. In some, the prevalence of three quartered cows approaches 50%. Clearly, these herds have not yet met the challenge of preventing environmental mastitis, yet they maintain bulk tank somatic cell count 200,000-250,000/ml.

The reservoirs for environmental mastitis pathogens are the manure and the bedding. Microbes living in these reservoirs gain entry into the udder through the teat canals during the housing period, or during milking. So, on the face of it, the challenge seems straight forward – keep the cow clean in the housing environment and attach the milking units to clean dry teats. It is no surprise that herds that implement this approach can almost eliminate clinical mastitis as a concern for the farm. We have focused on milking cow preparation and made significant improvements in many herds, identifying clean teats at unit attachment as being imperative for the production of high quality milk. In the housing environment, we have designed facilities to keep cows clean, dry and comfortable – with similar success.

This paper discusses the elements of barn design that are integral to keeping udders clean, bedding bacterial counts low and cows comfortable. This has been the focus of our outreach program; 'The Dairyland Initiative' which was launched in 2010 and now has almost 4,500 users worldwide. More details on housing recommendations are available on the site at: https://thedairylandinitiative.vetmed.wisc.edu/

In this article, I will specifically focus on three critical areas:

1. Stall Design
2. Stall Bedding
3. Stall Microclimate Control

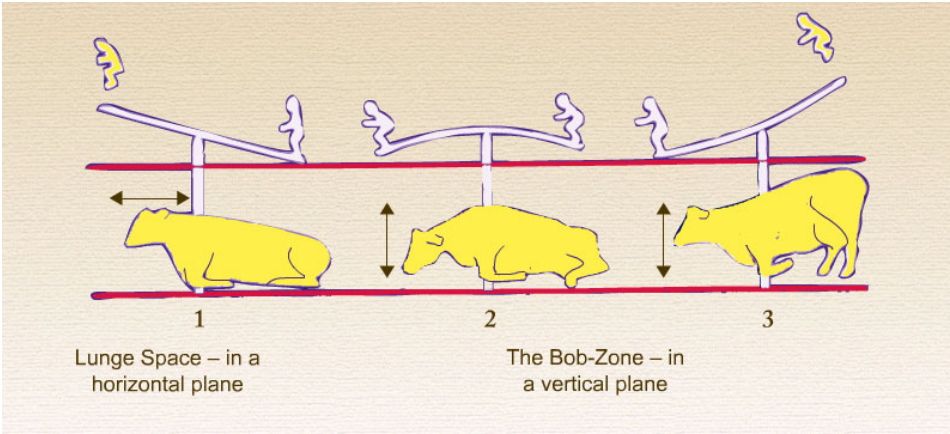
Stall Design

Dimensions

Freestalls should be appropriately sized to accommodate the resting imprint of the cows using them, and permit sufficient space for the cow to lunge forward when rising and lying down, without movement obstruction. Critical dimensions for freestall design include width (measured on center between divider loops), length (from the rear point of the curb to the furthest forward point the cow can lunge to) and the distance from the rear curb to the brisket locator.

Producer concerns related to stall hygiene when larger dimensions are suggested have not been realized when the stall is correctly designed and the correct dimension choice made based on the guidelines above. These recommendations allow the cow to rise and lie down as naturally as possible, without obstruction to the lunge or bob of the head, or the forward stride of the forelimb (Figure 1).

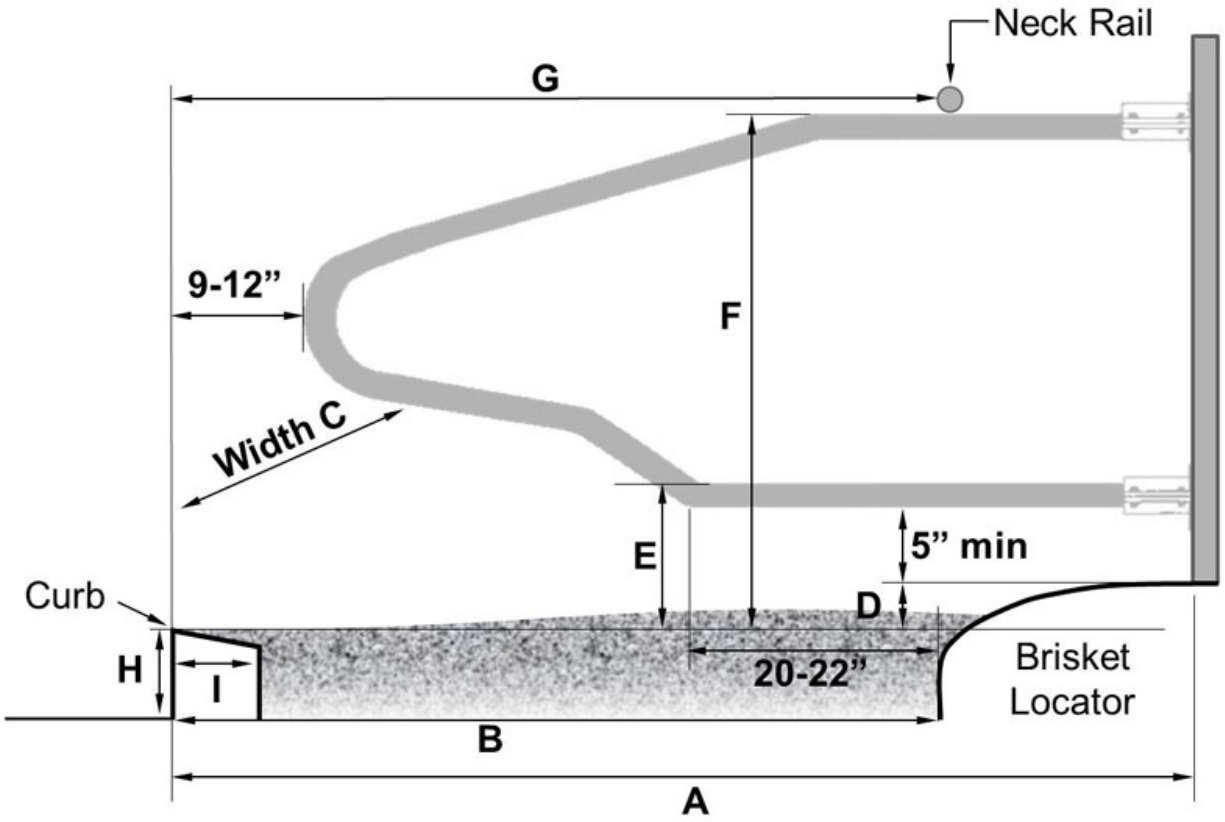
Figure 1. Note the lunge and bob space required by the cow as she rises. This allows the cow to transfer weight from her rear limbs, facilitating rising.



Target stall dimensions for cows of a range of sizes are given in table 1 for ideal stalls in pens without mixed age groups. The diagram identifies the location of the measurements and stall widths are measured on center.

Typical lunge space for a mature Holstein cow would be an additional 3 to 4 feet (91 to 122 cm) beyond the resting length, making the total required stall length against a side wall around 10 feet (3 m) for a mature Holstein cow. In head-to-head stalls, a platform of 16 to 18 feet (4.9 to 5.5 m) is recommended to allow lunging to the front, while avoiding the cow in the opposing stall.

Table 1. Target freestall dimensions for a range of body sizes



Stall Dimension (inches)	Body Weight Estimate (lbs)				
	1000	1200	1400	1600	1800
Total stall length facing a wall (B)	96	96	108	120	120
Outside curb to outside curb distance for head-to-head platform (B)	192	192	204	204	216
Distance from rear curb to rear of brisket locator (C)	64	66	68	70	72
Center-to-center stall divider placement (Stall width) (A)	44	46	48	50	54
Height of brisket locator above top of curb (loose bedded stall) or mat/mattress surface (G)	3	3	4	4	4
Height of upper edge of bottom stall divider rail above top of curb (loose bedded stall) or mat/mattress surface (H)	11	11	12	12	12
Height of neck rail above top of curb (loose bedded stall) or mat/mattress surface (J)	44	46	48	50	52
Horizontal distance between rear edge of neck rail and rear edge of curb for mattress stalls (E)	64	66	68	70	72
Rear curb height (M)	8	8	8	8	8
Rear curb width (loose bedded stalls) (D)	6	6	6	6	6

**E in deep, loose-bedded stalls is less than in mattress stalls to encourage cows to stand with rear feet in alley instead of on stall base in order to reduce contamination of the bedding*
Brisket Locators

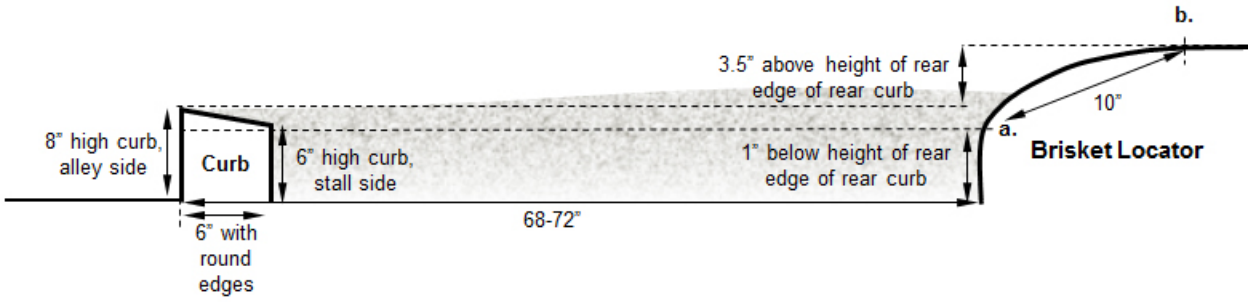
The resting space in front of the cow is defined by a brisket locator, the purpose of which is to assist in the alignment of the cow when lying in the stall, so manure lands in the alley rather than on top of the stall if the cow defecates. When located too far from the rear edge of the curb, cows will lie too far forward and soil the rear of the bed. When located too near the rear curb, cows will tend to lie more diagonally across the stall.

We must not obstruct the forelimb as it is thrust forward to support the weight of the cow during the rising movement. Brisket locators must therefore be no higher than 4 inches (10 cm) above the stall surface so the cow can get her leg over the top of the locator. An obstruction higher than this forces the cow to get up by thrusting her forelimb directly downwards, rather than out in-front of her, making rising more difficult.

The traditional brisket 'board' was a 2x10-inch (5x25-cm) board which was sometimes used as a form to pour the concrete curb. Wooden briskets may still be used provided they are correctly located, as described above. Round PVC or fiber glass pipes have been utilized, with various fixing designs. Cows may lie over the top of these smooth rounded designs creating problems with cow placement. Where the brisket locator is fixed to the lower divider rail, it is very common to see the locator mounted too high. Care should therefore be taken when using this kind of fixing to make sure that it is adjustable. They will also require some maintenance as they will rust in sand bedding and loosen over time.

Recently, an alternative brisket locator design has been developed (Figure 2). The design utilizes concrete which slopes gently from the target locator position up to a height of 3.5 inches (9 cm) above the rear curb. The slope is distinct enough to position the cow, while being shallow enough for the cow to thrust her foot securely against it when rising. This concrete may also be used to anchor vertical posts used for divider loop mounting.

Figure 2. Concrete brisket slope design



Stall Divider Loop Design

The freestall divider has a number of important functions, including:

1. Defining the lateral limits of the resting space
2. Facilitating lying direction of the cow – straight rather than diagonal
3. Permitting or preventing side lunge
4. Determining the height of the neck rail

Obviously it is preferable if the divider performs these functions without injuring the cow and for this section we will refer to dimensions suitable for a 1400-1600lb typical Holstein cow. The most important part of the divider is the lower rail. This rail's purpose is to suggest to the cow just enough, but not too much, where to lie down and it must allow her to rise without obstruction or risk of injury.

The height of the lower rail is critical. It must allow for at least a 5 inch (13 cm) gap between the lower edge of the bar and the top of any brisket locator. This will prevent any front leg entrapment below the rail. It must also be high enough, but not too high! Low divider rails allow cows to rise with their front legs over the lower rail – leading to entrapment and a cow caught in the middle of the loop. High divider rails will at some point prevent side lunge into the adjacent stall, unless they are so high that the cow lunges below them – such as the Michigan or dog-bone loop design, which are not recommended.

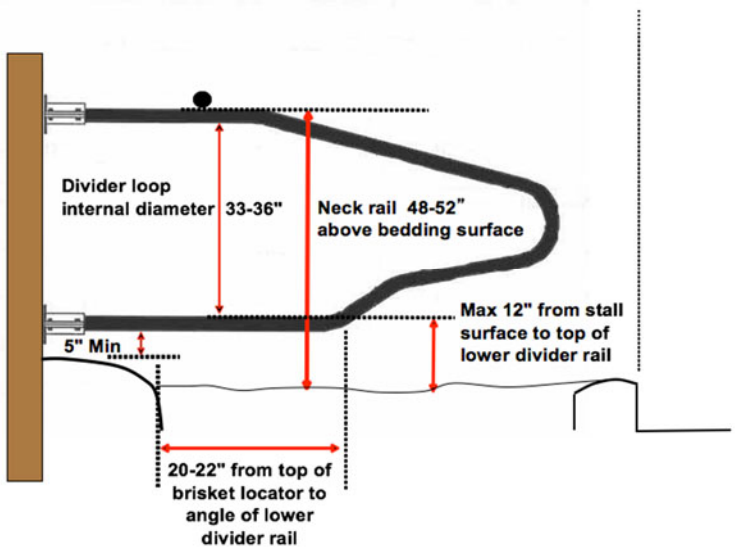
If the stall is long enough to permit front lunge, a lower rail that prevents side lunge is perhaps permissible. However, on head to head stalls, cows in the opposite stall may pose a social obstruction to a subordinate cow – creating a desire to side lunge even when there is space provided. For that reason, our preference is to use a divider that allows side lunge, however we do not believe that we should use a divider and stall design that only permits side lunge. Cows that front lunge lie straight in the stall and stay cleaner.

The height of the lower rail above the level stall surface (or rear point of the curb in a deep loose bedded stall) should be between 11-12 inches (28-30 cm) for most mature Holstein cows. The rail should be level and not angle down to the surface (like the green or freedom stall divider) – this type of loop will promote side lunge and diagonal lying as the cow takes advantage of the lower rail height at the front of the stall. Loops designed for side lunge (Michigan and Dog-bone designs) - where the cow lunges below the lower rail also promote diagonal lying, dirty stalls and medial hock injuries, and are therefore not recommended. Similarly loops where the lower rail starts level, but bends down to mount onto the top of the stall surface in front should also be avoided, as these also invite the cow to lie too far forward and side lunge.

The lower rail should extend back toward the rear curb enough to suggest where the cow should lie straight in the stall, but not so far back that the rail impacts the hooks and rump of the cow as she lies down, before it angles upward and out of the way. From measurements we have taken we recommend that the angle be 20-22 inches (51-56 cm) toward the rear curb back from the brisket locator. Since the location of the brisket locator will range typically from 68-72 inches (173-183 cm) from the rear curb and stall length may vary, we need different sized loops for different sized stalls!

We prefer dividers that are loops with a level or angled upper rail. The angle allows the cow to pivot her head out of the stall when she is leaving a little easier. The open diameter of the loop determines the height of the neck rail. A distance of 33-36 inches (84-91 cm) from the upper edge of the lower rail to the lower edge of the upper rail should locate the neck rail at the target height of 46-52 inches (117-132 cm) – depending on the size of the cow. Lastly, the rear limit of the divider should be 9-12 inch (23 to 30 cm) inside the rear curb – close enough to prevent cows from walking along the back of the stalls, but not so close that it gets hit by passing machinery.

Figure 3. Vertical positioning of the divider loop for mature 1400-1800 lb dairy cattle



To transfer as much weight as possible from the hind-limbs when rising and lying down, the cow moves her head downward toward the ground at the end of the lunge, and then moves her head upward as she completes the rising and lying movement sequence. This area from the stall surface to a height of around 38 to 40 inches (97 to 102 cm) at the end of the lunge is called the 'bob zone' (Figure 1), and it must be free of obstruction. We therefore do not recommend accumulation of mounds of bedding, transverse mounting bars or deterrent bars which impinge on cow head movement in this area.

Neck Rail

The neck rail provides lateral stability to the stall dividers, while helping position the cow in the stall while she is standing. Neck rails have less impact on the lying position of the cow. Proper standing position limits the amount of manure on the rear of the stall.

Correct location of the neck rail differs slightly between deep loose bedded stalls with a raised curb and mattress surface stalls.

In stalls with mattress surfaces, the correct neck rail location is directly above an appropriately located brisket locator at a height of 48 to 50 inches (122 to 127 cm) for a mature cow. It is suggested that once located here, standing position of the cows should be observed and the exact location of the rail modified so the majority of cows in the pen can stand squarely on the stall platform with all four feet, while manure lands in the alley.

In deep loose bedded sand stalls with a raised rear curb, correct location of the neck rail is problematic. When located directly above a correctly positioned brisket locator, too much manure and urine is deposited in the stall. This is because the rear curb design and dimensions influence where the cow stands in the stall. Cows do not like to stand on a raised, rounded or sloped rear curb, and instead will stand diagonally across the stall, with their rear feet inside the curb on the soft bedded surface. From this position, manure and urine will be deposited into the

bedding. We must therefore adjust the location of the neck rail in such a stall. In stalls with the neck rail 46 to 50 inches (117 to 127 cm) above the stall surface, it can be moved back from above the brisket locator towards the rear curb a distance of about 6 inches (15 cm); equivalent to the width of an appropriately designed rear curb. This will force cows to take a step back and perch in the stall when rising.

Stall Bedding

Dairy producers worldwide must decide between two main types of resting surface; deep loose bedding or a mattress type surface. Thin rubber mats or concrete surfaces with little bedding have no place in adult cattle housing and should not be used. Bedding material choices are secondary and usually consist of sand, manure solids or other organic type bedding (straw, sawdust, shavings, hulls, paper, peat etc).

Currently, 70% of freestall facilities in Wisconsin use deep loose bedding, 60-64% use sand, while the remainder use manure solids, or other organic materials (Brotzman et al., 2015). Only 30-32% of herds use a mattress or mat - most commonly a rubber crumb filled type mattress. In a recent survey of Wisconsin herds shipping more than 25,000 lb milk per day, 60% of herds used inorganic bedding and produced 2,401 lb (1,091 kg) more milk per cow per year than the 9% of herds bedding with manure solids and 1,859 lb (845 kg) more milk than the 19% of herds using another type of organic bedding (Rowbotham and Ruegg, 2016). These inorganic bedding users also had lower somatic cell counts than the other two groups (198,000/ml inorganic vs 248,000/ml manure solids and 220,000/ml organic bedding).

Sand is a granular material consisting of finely divided rock and mineral particles. Dry matter (DM) is usually 94-97%, (drier is better) and organic matter (OM) content is usually <4%. Granular size varies. Very coarse sand drains moisture from the surface of the bed well, but may be very abrasive and lead to excessive hoof wear, while very fine sand will trap moisture and organic matter which may fuel bacterial growth and create associated risk for mastitis. In general, we select sand that is neither too coarse, nor too fine for optimal comfort and udder health. For reclamation systems a coarser sand is usually selected than for fresh sand systems, and we suggest using sand that has had a sieve analysis performed. Separation systems perform optimally with a mason or concrete type sand.

Table 2. The ASTM standards for Mason (a.k.a. Number 8 sand) and Concrete Sand sieve analysis

U.S. Sieve Number	Sieve Opening (in)	Mason Sand ASTM C144		Concrete Sand ASTM C33	
		Min	Max	Min	Max
3/8	0.375	100	100	100	100
4	0.187	100	100	100	95
8	0.0937	100	95	100	80
16	0.0469	100	70	85	50
30	0.0234	75	40	60	25
50	0.0117	35	10	30	10
100	0.0059	15	2	10	2

Sand DM, OM and bacterial load may be monitored regularly by sampling grossly uncontaminated sand from the rear of a random selection of stalls within a pen.

Suggested targets for ideal sand would be:

DM >95%
OM<4%
Fresh bedding total bacterial count: < 5,000 CFU/mL
Used bedding total bacterial count: < 1-2 million CFM/mL (mostly streptococci)
Coliform count in used bedding: < 100,000 CFM/mL

Whatever type of sand is used, the compaction zone below the surface where the sand is becoming as hard as concrete should be monitored. If this gets to within 1 to 2 inches (2.5 to 5 cm) of the point of the rear curb, it is time to remove the rear third of the bed and replace with fresh uncontaminated sand. Adding fresh sand twice a week is usually sufficient to maintain reasonable fill, and grossly contaminated sand should be removed each milking. Note: Only sand that is contaminated with organic matter needs to be removed. An easy stall-side test is to ball the sand in your had and juggle it. If the ball stays in a ball the sand needs to be removed. If it breaks up easily it can stay in the stall.

Leveling and aerating the bed is essential for successful sand stall management. Level by redistributing the sand from beneath the divider loops and aerate the top 3-4 inches of the bed. This may be done by hand in smaller herds. The rake user must be taught to drag sand diagonally from under the divider loops back to the rear of the stall flush with the rear curb after removing the wet contaminated sand. In larger herds a mechanical leveler can be used.

Figure 4. Diagonal redistribution of sand from beneath the loops to the stall bed

The typical range of sand use rates is typically 20 to 80 lbs (9.1 to 36 kg) sand per stall per day, with an average of ~ 50 lbs (23 kg). Herds must decide whether or not to attempt sand reclamation from the manure. This decision will depend on the cost of sand, herd size, land availability, soil type and nutrient management, and other economic factors. Sand settling lanes are in operation in herds as small as 120 cows, but in general, herds with more than 800-1000 cows tend to recycle sand. When sand is correctly recycled, cows are at no greater risk for udder health problems.

Stall Microclimate Control

The importance of ventilation and control of relative humidity in calf barns has been recognized for some time in regard to its role in respiratory disease. High humidity environments enhance air borne pathogen survival and it is likely that in adult cow barns, high humidity does little to help dry recycled bedding materials nor control bacterial growth in them – yet ventilation has received little attention in mastitis control strategies.

I view the stall as a microclimate within the cow barn where, particularly during the summer, we must improve air movement and reduce relative humidity. High producing cows produce more heat than lower producers, and the dairy industry is trending toward the construction of more mechanically ventilated facilities, in an attempt to ameliorate heat stress risk. In these barns, use of wet recycled sand and manure solid bedding has become very challenging. This material fails to dry and is at risk for maintaining very high bacterial loads.

In our facility designs, we prioritize fast moving air in the resting space as the number one design priority. We recommend at least 400 ft/min (4.5 mph) (2 m/s) for optimal cooling air speed at cow level.

The two main ways of achieving this goal are with baffles or fans. Baffles are ideally suited for cross ventilated barns where a single baffle can redirect air below it over the stalls for the entire length of the barn – especially when we have a head to head stall layout. The baffle forces air down into the stalls for about the length of the stall platform. However, in the winter, baffles serve to trap stale air between them and reduce the efficiency of winter ventilation – we therefore prefer retractable curtain baffles, rather than solid permanent structures, so we can pull them out of the way in the winter. Baffles are ineffective in tunnel ventilated barns since they only impact a few cows lying below them across the width of the barn, so the alternative to providing fast moving air is the use of fans.

To optimize the number of cows exposed to cooling air speeds of about 400 ft/min (4.5 mph) (2 m/s), most 48 to 50-inch (122 to 127 cm) panel fans need to be spaced 20 to 24 feet apart (6.1 to 7.3 m), while 72-inch (183 cm) cyclone fans need to be spaced 60 feet (18.3 m) apart. These resting space fans may also be used at low speeds in the winter to facilitate air flow across or along the barn if they are fitted with variable frequency drives. These fan spacings are generally closer together than we have previously seen recommended. Installers must be aware that throw distance and electrical usage varies significantly from fan to fan and adapt accordingly to optimize air distribution. We have also used positive pressure fans to deliver air directly into the stalls using a tube delivery system. Current fan specifications limit this type of installation to ~ 100 stall pen.

Once we have ensured adequate air movement in the resting space, all we need to do is make sure that the rest of the barn ventilates to a reasonable number of air changes per hour – typically 4 ach in the winter, 15-20 ach in transitional periods and 40-60 ach in the summer. I believe this approach is essential if we are to control humidity levels and bacterial growth in bedding.

Conclusions

The maintenance of a clean dry comfortable environment for dairy cattle in confinement housed facilities is a challenge. Excellent udder preparation can offset some of the deficiencies we see in barn design, but not all. Bacterial challenge to the teat end can be reduced through good stall design, excellent bedding management and control of the stall microclimate. In this article I have attempted to lay out our current recommendations in these areas and more information can be sourced at http://thedairylandinitiative.vetmed.wisc.edu.

References

Brotzman, R.L., D. Dopfer, M.R. Foy, J.P., Hess, K.V. Nordlund, T.B. Bennett, and N.B. Cook. 2015. Survey of facility and management characteristics of large, Upper Midwest dairy herds clustered by Dairy Herd Improvement records. J. Dairy Sci. 98:8245-8261.

Rowbotham, R.F., and P.L. Ruegg. 2016. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J. Dairy Sci. 98:7865-7885.

Bedding, Bugs and Teats – Evaluating Environmental Risks of Intramammary Infection

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Introduction

In the U.S., the majority of milk is produced on farms that contain greater than 500 milking cows (http://www.nass.usda.gov/Quick_Stats) and the presentation of mastitis has evolved as farm size had increased. Larger dairy farms have greater adoption of modern management practices that reduce transmission of subclinical intramammary infections (Rodrigues et al., 2005, Rowbotham and Ruegg, 2015). These improvements have contributed to control of *Staph. aureus* and near eradication of *Strep. agalactiae* and resulted in considerable decreases in bulk tank SCC (Figure 1). While intensification has resulted in reduced bulk tank SCC, mastitis remains a significant challenge for many dairy farms. Increased animal densities and changes in dairy housing (Ericsson Unnerstad et al., 2009) have increased potential exposure to opportunistic intramammary pathogens that often present with mild clinical signs, and national surveys have indicated that the rate of clinical mastitis has consistently increased (Figure1). In most herds, the majority of clinical cases are caused by opportunistic pathogens that originate from the environment (Oliveira et al., 2013).

Figure 1. Bulk Tank SCC and Clinical Case Rate in U.S. Dairy Herds

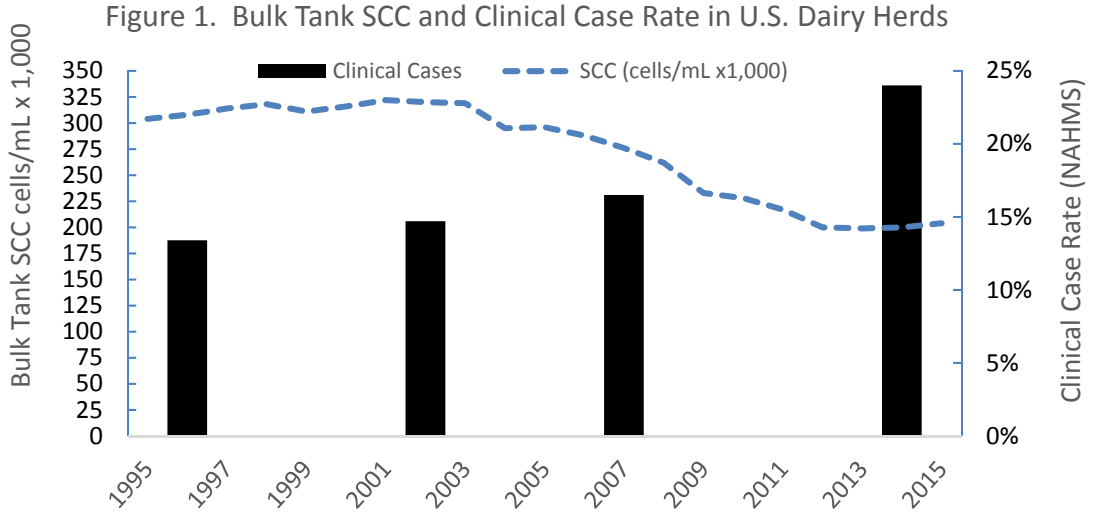


Figure 1 data from: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms

Exposure to opportunistic bacteria usually occurs when teats of susceptible cows come in contact with bacteria that are found in housing areas. Common pathogens include both Gram-negative bacteria (such as *E. coli* and *Klebsiella* spp.) and Gram-positive bacteria (such as *Strep. uberis* and other *Streptococcal* like organisms). Opportunistic pathogens tend to be less adapted to survival in the udder and intramammary infection often triggers sufficient inflammation to result in visually apparent mild or moderate clinical signs. Bedding materials, and moisture or manure

in animal walkways are common reservoirs and controlling environmental mastitis is based on reducing exposure of teats of the most susceptible cows. The objective of this paper is to briefly review selected risk factors that are associated with exposure to environmental mastitis pathogens.

Exposure to Bacteria in Bedding

Manure handling, type of bedding and stall maintenance all have significant impacts on exposure of teats to mastitis pathogens. As farm size increases, options for management of waste materials (bedding residue and manure) change. To effectively manage costs and environmental restrictions, larger farms use a wide variety of bedding materials. While many bedding materials initially have relatively low bacterial populations, organic matter in some bedding contains nutrients that support bacterial growth and results in exposure of teats to a great variety of potential mastitis pathogens. This is especially true of recycled manure which is very rich in nutrients that support growth of fecal organisms. A recent observational study of large Wisconsin dairy farms demonstrated lower RHA, greater SCC, more treated cows and a greater proportion of cows with non-functional quarters in herds that used manure based bedding as compared to herds that used sand (Rowbotham and Ruegg, 2015). In general, the number of Gram-negative bacteria (often associated with shorter duration infections and occurrence of clinical mastitis) is greater in organic bedding materials (such as recycled manure solids) as compared to new sand bedding (Table 1). However, the number of opportunistic Gram-positive bacteria (often associated with longer duration subclinical infections) can be quite significant recycled sand (Table 1) and intramammary infections with these organisms may contribute to increased BTSCC.

Table 1. Number of bacteria in bedding and teat skin swabs from selected studies (Hogan and Smith, 1997 (Study #2), Hogan et al., 1999 (Study #3), Rowbotham and Ruegg, 2016 (Study #1)) that used the same laboratory methods (log cfu/gram of bedding or log cfu/teat swab)

					Manure solids		
Bacterial type	Study # & day in stall	Source	New Sand	Recycled Sand	Deep bedded	On top of mattresses	Sawdust
Coliform	1 (day 3)	Bedding	3.6[a]	4.0	5.6[a]	4.1	
		Teat skin[b]	1.1	1.5	2.4	1.3	
	2 (day 2)	Bedding					6.0
		Teat skin					1.7
	3 (day 6)	Bedding				6.8	6.1
		Teat skin				2.5	2.5
Klebsiella	1 (day 3)	Bedding	2.6	3.3	5.0	2.9	
		Teat skin	0.4	1.4	2.1	0.7	
	2 (day 2)	Bedding					5.7
		Teat skin					1.6
	3 (day 6)	Bedding				6.2	5.9
		Teat skin				2.2	2.1
Strep spp.	1 (day 3)	Bedding	6.9	7.2	7.1	8.2	
		Teat skin	4.3	4.8	3.8	5.1	
	2 (day 2)	Bedding					7.0
		Teat skin					4.5
	3 (day 6)	Bedding				7.8	7.0
		Teat skin				4.0	3.9

[a]Expressed as Log_{10} values: for example $10^{3.6} = 3{,}625$ cfu/gm (median value) while $10^{5.6} = 2{,}250{,}000$ cfu/gm median; [b]teat skin before sanitation

The number of bacteria recovered from teat skin is typically 2-3 log units (100 to 1000 times) less than that found in bedding (Table 1), indicating potentially greater risk of infection for quarters exposed to bedding that contains greater quantities of bacteria. A linear relationship between exposure to bacteria in bedding and rate of Gram-negative clinical mastitis during lactation has been demonstrated but that association was relatively weak and the authors of the study cautioned that <16% of variation in clinical mastitis rate could be attributed to differences in bedding bacterial count (Hogan et al., 1989). Exposure to bacteria alone doesn't necessarily result in intramammary infection. For all infectious diseases, exposure to a pathogen is necessary for infection, but mastitis is a multifactorial disease and other risk factors are needed for exposure to result in mastitis. Factors that influence the risk of infection with opportunistic pathogens include management factors such as design and usage of stalls, management of bedding (including particle size and content of moisture and organic matter), adequacy of milking procedures and gentleness of milking. Important cow-level factors include anatomical characteristics of the udder and teats. While is exposure is important, risk of intramammary infection is also influenced by the ability of the cow to mount an effective and rapid immune response after bacteria have penetrated the teat orifice.

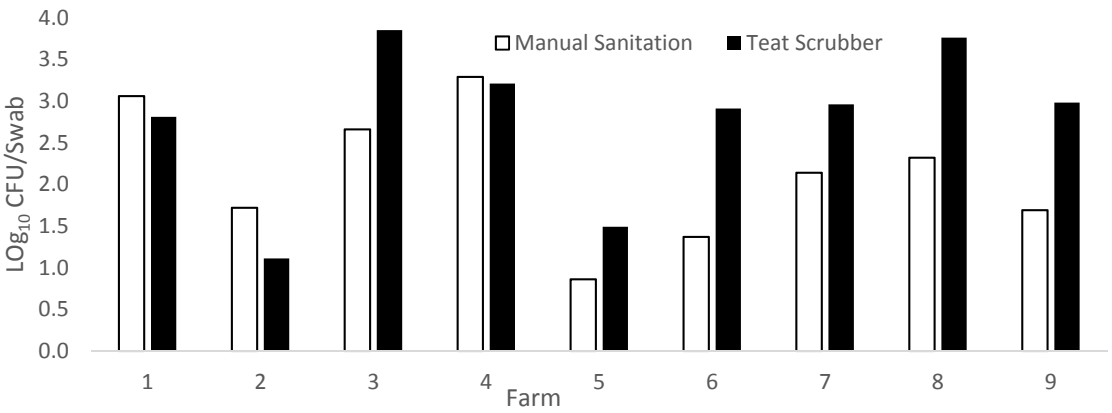
Teats are exposed to bacteria found in bedding and the magnitude of risk is related to the number of bacteria. It is logical that reducing bedding conditions that are favorable to microbial growth will reduce risk of intramammary infection and strategies to reduce bacterial growth have been

evaluated. The addition of disinfectant additives to bedding has been demonstrated to have only a short-term impact and optimal management of organic bedding materials to minimize growth of bacteria is not well defined (Hogan et al., 2007, Hogan and Smith, 2012). Bacterial growth requires nutrients, moisture and an appropriate temperature. Data collected twice monthly over 8 months, from 9 Wisconsin dairy farms that utilized separated solids (7 using digesters) indicated that dry matter of solids (prior to use) ranged from 26 to 31%, and when driers were used, increased to about 37-39% (personal communication, Becky Larson, UW Madison). On many farms, used bedding is drier than fresh bedding (Sorter et al., 2014) and when bedding is added more frequently to stalls, it may actually provide more moisture and perhaps encourage microbial growth. Dry matter of unused and used manure bedding samples collected from 38 dairy farms in the Upper Midwest were 28% and 50%, respectively (Husfeldt et al., 2012). For virtually any bedding type, reducing moisture is advantageous in reducing both bacterial growth and increasing lying time (Fregonesi et al., 2007) and effort should be made to provide as dry as possible lying surface for cows.

Impact of Teat Sanitation on Teat Skin Bacteria

Prevention of new intramammary infection is the usual outcome evaluated in teat dip efficacy trials. It is well established that properly performed pre-milking teat sanitation is effective in reducing the incidence of intramammary infections caused by opportunistic organisms by at least 50% (Pankey et al., 1987, Oliver et al., 1993). However, authors of a meta-analysis evaluating factors that influence teat dip efficacy recently concluded that experimental design had a very large impact on the outcome of efficacy trials (Enger et al., 2016). Initial evaluations of the ability of teat dips to reduce teat skin bacterial counts are often performed using controlled experiments that minimize variation in application, concentration and milking protocols that exist on real dairy farms. Using a controlled experiment with excised teats, 2 to 5 log reductions in teat skin bacteria have been reported for a variety of teat dips (Enger et al., 2015). While large reductions in teat skin bacteria are often noted in experiments, on real farms the ability to successfully sanitize teats varies tremendously, even when using a "proven" teat dip. The ability to successfully reduce teat skin bacterial counts is influenced by many factors including: initial amount of teat skin bacterial contamination, environmental conditions, willingness of cows to tolerate pre-milking sanitation, design of milking parlors, ancillary milking procedures used as part of the parlor work routine, training and compliance of milking personnel and compliance with teat dip labels for storage and/or on-farm formulation (for dips that are mixed on farms). Baumberger et al., (2016) evaluated reduction in numbers of Gram-negative bacteria on teat skin using 2 methods performed on 9 separate farms and noted considerable variation in reduction of teat skin bacteria among farms (Figure 1).

Figure 1 - Reduction in Gram-negative Teat Skin Bacteria after Pre-Milking Sanitation using 2 Different Methods (from Baumberger et al., 2016)



In this study, even though manual preparation was performed by a single researcher (across all farms) using a standard procedure and consistent disinfectant, reduction in Gram-negative bacteria, varied from 1 to 3 log CFU. Reasons for the differences are unknown but likely include differences in bedding types and initial load of bacteria on teat skin. Variation in bacterial reduction using the teat scrubber was even greater (ranging from 1 to 3.5 log CFU) and was influenced by use of ancillary procedures (such as pre-wipe) and concentration of chlorine dioxide supplied to the teat scrubber in the on-farm system (Figure 1, (Baumberger et al., 2016). Among herds, concentration of chlorine dioxide varied from 50-850 ppm and herds that used <500 ppm had less effective reduction in teat skin bacterial counts. While use of "scientifically proven" pre-milking teat sanitizers is always recommended, we need to recognize that on-farm conditions can have a large effect on the ability to satisfactorily reduce bacterial populations of teats, and this is especially true for farms that have housing systems that expose teats to large number of bacteria.

Cow Level Risk Factors

Reducing risk of opportunistic intramammary infections is based on reducing exposure to potential pathogens but risk of developing mastitis is not equal among animals because different groups of cattle have differing abilities to withstand environmental challenges. The ability to resist and respond to infection is influenced by both stage of lactation and parity. As compared to older animals, cows in first and second lactation have reduced risk of developing clinical mastitis caused by opportunistic pathogens (Zadoks et al., 2001, Pantoja et al., 2009, Pinzon-Sanchez and Ruegg, 2011). Stage of lactation is also a risk factors for development of clinical mastitis and the disproportionate occurrence of clinical mastitis in early lactation is a hallmark of mastitis caused by environmental pathogens (Oliveira et al., 2013). It is well documented that leaking milk, high daily milk yield and reduced immunological capabilities are associated with increased risk of clinical mastitis (Schukken et al., 1990) and all of these characteristics are more common in early lactation. While exposure to opportunistic environmental pathogens can occur throughout the lactation cycle, cows initiating lactation are less able to withstand exposure to microorganisms because of innate immune suppression.

Anatomical characteristics of the udder and teat are known risk factors for intramammary infection. Cows with larger udders are at increased risk of intramammary infection as are cows

with udder hygiene scores that indicate dirtier udders (scores 3 or 4 on a 4-pt. scale) (Barkema et al., 1999, Schreiner and Ruegg, 2003). Udders become dirty as a consequence of a number of routine management decisions. Risk factors for "dirty udders" were evaluated on 79 commercial Wisconsin dairy farms (Salgado and Ruegg, data unpublished). The farms included 11,200 lactating cows housed in both freestalls (n = 51 herds) and tie stall barns (n = 28). There was no difference in the proportion of clean UHS (77%) based on type of facility. For animals housed in tie stalls, the risk of dirty udders was increased 1.5 times when stalls were cleaned <2 times per day, 4.5 times when stall beds were scored as dirty, and >10 times when a large proportion of the cows had loose manure. For animals housed in freestalls, the risk of dirty udders was increased 1.8 times when organic bedding materials were replenished less than daily, 4 times when stall beds were scored as "dirty," >10 times when a large proportion of the cows had loose manure, 2.5 times when cows had access to outdoors and >10 times as barns were increasingly overstocked. This data reinforces the role of facility management and cow comfort in reducing risk of environmental mastitis.

Specific characteristics of teats have also been associated with increased risk of clinical mastitis. While teats of U.S. Holsteins are considerably shorter today as compared to previous decades (Guarin and Ruegg, 2016, Guarin et al., 2017), as cows age, teat shape changes and teats of cows in $\geq 3^{rd}$ parity tend to be longer with wider apexes and thus have potentially less ability to withstand exposure to opportunistic pathogens. There is some evidence that increased width of the teat apex is associated with increased risk of both clinical and subclinical mastitis(Guarin and Ruegg, 2016, Guarin et al., 2017). These risks seem to be greatest for front teats. In a recent single herd study, we observed a 20% increased risk of clinical mastitis associated with each 1mm increase in diameter of the front teat apex (Guarin and Ruegg, 2016) . The occurrence of severe hyperkeratosis is also a risk factor for clinical mastitis (Neijenhuis et al., 2001, Guarin et al., 2017). While mild hyperkeratosis scores (N, S and R) have not been associated with increased risk of mastitis, teats most severely affected with hyperkeratosis (score of VR) have increased risk of intramammary infection and minimizing the proportion of teats with severe hyperkeratosis should be a priority of milking managers.

Conclusion and Recommendations

For decades, the role of herd management in controlling environmental mastitis has been well recognized and control is based on reducing the likelihood that teats of susceptible cows will be exposed to opportunistic pathogens. Udder health programs should be proactively focused on prevention of initial infections because occurrence of the first case of mastitis is a strong predictor of future cases (Pinzon-Sanchez and Ruegg, 2011). Thus, efforts should be directed at providing an optimal environmental for the highest risk cows. This knowledge can be used to make practical management decisions. Barns and pens used for transition and fresh dairy cows should be low density (providing no less than 100 ft^2 ($10m^2$) of dry lying space per cow), utilize low moisture bedding materials that minimize microbial growth (clean sand is best) and be designed to provide maximum cow comfort. Herds that are dependent on using bedding that supports growth of Gram-negative bacteria may consider milking a greater proportion of lower-risk cows (first and second lactation, cows with smaller udders and narrower teats apexes) and consider genetic selection of cows with characteristics that enhance the ability to resist intramammary infection (cows that don't leak milk and have smaller udders). Cows that have recurrent clinical cases should be culled. In all instances, milking routines should be designed to

appropriately sanitize teats and minimize the risk of developing hyperkeratosis. Sufficient management of the milking parlor should be provided to ensure compliance with well-designed milking routines that effectively sanitize teats and allow for consistent detection of mild clinical cases.

References

Barkema, H.W., Y.H. Schukken, T.J.G.M. Lam, M.L. Beiboer, G. Benedictus, and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. J Dairy Sci 82(8):1643-1654.

Baumberger, C., J.F. Guarin, and P.L. Ruegg. 2016. Effect of 2 different premilking teat sanitation routines on reduction of bacterial counts on teat skin of cows on commercial dairy farms. J Dairy Sci 99(4):2915-2929.

Enger, B.D., L.K. Fox, J.M. Gay, and K.A. Johnson. 2015. Reduction of teat skin mastitis pathogen loads: Differences between strains, dips, and contact times. J Dairy Sci 98(2):1354-1361.

Enger, B.D., R.R. White, S.C. Nickerson, and L.K. Fox. 2016. Identification of factors influencing teat dip efficacy trial results by meta-analysis. J Dairy Science 99(12):9900-9911.

Ericsson Unnerstad, H., A. Lindberg, K. Persson Waller, T. Ekman, K. Artursson, M. Nilsson-Ost, and B. Bengtsson. 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet Microbiol 137(1-2):90-97.

Fregonesi, J.A., D.M. Veira, M.A.G. von Keyserlingk, and D.M. Weary. 2007. Effects of bedding quality on lying behavior of dairy cows. J Dairy Sci 90(12):5468-5472.

Guarin, J.F., M.G. Paixao, and P.L. Ruegg. 2017. Association of anatomical characteristics of teats with quarter-level somatic cell count. J Dairy Science, available online: https://doi.org/10.3168/jds.2016-11459.

Guarin, J.F., and P.L. Ruegg. 2016. Short communication: Pre- and postmilking anatomical characteristics of teats and their associations with risk of clinical mastitis in dairy cows. J Dairy Sci 99(10):8323-8329.

Hogan, J.S., V.L. Bogacz, L.M. Thompson, S. Romig, P.S. Schoenberger, W.P. Weiss, and K.L. Smith. 1999. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. J Dairy Sci 82(8):1690-1695.

Hogan, J.S., and K.L. Smith. 1997. Bacteria counts in sawdust bedding. J Dairy Sci 80(8):1600-1605.

Hogan, J., and K.L. Smith. 2012. Managing Environmental Mastitis. Vet Clin N Am-Food A 28(2):217-2024.

Hogan, J.S., K.L. Smith, K.H. Hoblet, D.A. Todhunter, P.S. Schoenberger, W.D. Hueston, D.E. Pritchard, G.L. Bowman, L.E. Heider, B.L. Brockett, and H.R. Conrad. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J Dairy Sci 72(1):250-258.

Hogan, J.S., S.L. Wolf, and C.S. Petersson-Wolfe. 2007. Bacterial counts in organic materials used as free-stall bedding following treatment with a commercial conditioner. J Dairy Sci 90(2):1058-1062.

Husfeldt, A.W., M.I. Endres, J.A. Salfer, and K.A. Janni. 2012. Management and characteristics of recycled manure solids used for bedding in Midwest freestall dairy herds. J Dairy Sci 95(4):2195-2203.

Neijenhuis, F., H.W. Barkema, H. Hogeveen, and J.P.T.M. Noordhuizen. 2001. Relationship between teat-end callosity and occurrence of clinical mastitis. J Dairy Sci 84(12):2664-2672.

Oliveira, L., C. Hulland, and P.L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J Dairy Sci 96(12):7538-7549.

Oliver, S.P., M.J. Lewis, T.L. Ingle, B.E. Gillespie, K.R. Matthews, and H.H. Dowlen. 1993. Premilking Teat Disinfection for the Prevention of Environmental Pathogen Intramammary Infections. J Food Protect 56(10):852-855.

Pankey, J.W., E.E. Wildman, P.A. Drechsler, and J.S. Hogan. 1987. Field Trial Evaluation of Premilking Teat Disinfection. J Dairy Sci 70(4):867-872.

Pantoja, J.C.F., C. Hulland, and P.L. Ruegg. 2009. Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. J Dairy Sci 92(1):139-148.

Pinzon-Sanchez, C., and P.L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. J Dairy Sci 94(7):3397-3410.

Rodrigues, A.C., D.Z. Caraviello, and P.L. Ruegg. 2005. Management of Wisconsin dairy herds enrolled in milk quality teams. J Dairy Sci 88(7):2660-2671.

Rowbotham, R.F., and P.L. Ruegg. 2015. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J Dairy Sci 98(11):7865-7885.

Rowbotham, R.F., and P.L. Ruegg. 2016. Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls. J Dairy Sci 99(8):6594-6608.

Schreiner, D.A., and P.L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J Dairy Sci 86(11):3460-3465.

Schukken, Y.H., F.J. Grommers, D. Vandegeer, H.N. Erb, and A. Brand. 1990. Risk-Factors for Clinical Mastitis in Herds with a Low Bulk Milk Somatic-Cell Count .1. Data and Risk-Factors for All Cases. J Dairy Sci 73(12):3463-3471.

Sorter, D.E., H.J. Koster, and J.S. Hogan. 2014. Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in freestalls. J Dairy Sci 97(5):2965-2968.

Zadoks, R.N., H.G. Allore, H.W. Barkema, O.C. Sampimon, G.J. Wellenberg, Y.T. Grohn, and Y.H. Schukken. 2001. Cow- and quarter-level risk factors for Streptococcus uberis and Staphylococcus aureus mastitis. J Dairy Sci 84(12):2649-2663.

Aiming to Control Mastitis from Dry-off to Lactation

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Introduction

Except for some cases of hematogenous dissemination of *Mycoplasma spp*. there are 4 ways dairy cows acquire a bacterial intramammary infection (IMI): left front, right front, left hind, and right hind quarter (Johnson A, 2016 personal communication). Thus, the environment in which the cows live and are milked is of most importance in preventing IMM. The greatest incidence of clinical mastitis occurs within the first weeks following parturition (Fig 1), which overlaps with the transition period, a time of negative energy balance following the onset of lactation and immune dysregulation (Barkema et al., 1998, Green et al., 2002). Many of these infections were acquired during the dry period and become clinically apparent after parturition (Fig 2) and a shift in immune regulation. This short paper and talk will focus on prevention and treatment of IMI during the dry period while other presentations in this symposium discuss milking system management, facility design, and the lactating period. A full review of general strategies to control mastitis in the dry period is beyond the scope of this manuscript. The goal of mastitis control during the dry period is to minimize the incidence of IMI at the beginning of the next lactation. In order to achieve this, both prevention of new infections and clearing of existing infections must occur.

Physiology

Despite the challenges in udder health management during the non-lactating or dry-period period it also presents opportunity for mammary epithelial cells (MEC) to regress, proliferate, and differentiate to perhaps repair previously damaged secretory cells and build new ones such that milk production potential can be realized from a healthy gland. When milk is no longer removed from the gland, prolactin production diminishes, triggering apoptosis of mammary epithelial cells. Smith and Todhunter (1982) describe three phases of dry-period mammary gland physiology: active involution, steady-state involution, and colostrogenesis. The first phase represents a risky period for infection as the teat canal might remain open from intramammary pressure in the absence of good milking procedures. The kertain plug blocking the teat orifice provides some protection against new IMI during the steady-state phase. However, Dingwell and coworkers (2004) estimated that 5 to 23% of teats have an absence of long delay in plug formation. During colostrogenesis mammary secretions dilute protective factors (e.g. lactoferrin and active leukocytes, (Sordillo and Nickerson, 1988)) and if there is a kertatin plug it begins to break down leading to another increased risk period for bacterial invasion and colonization.

Immunology

The mammary gland innate immune defense is comprised of physical barriers such as the teat sphincter and keratin, soluble factors in mammary gland secretions including lactoferrin, complement, and defensins, and cellular defenses in the mucosa including tissue-resident cells and recruited leukocytes, i.e. MEC, monocytes, dendritic cells, macrophages, lymphocytes, neutrophils (PMN), mast cells, and natural killer cells. Stage of lactation, parity, metabolic

status, and immune function are associated with clinical severity of mastitis (Burvenich et al., 2003, Burvenich et al., 2007). After pathogen stimulation the inflammatory cascade leads to the recruitment of blood leukocytes to the infected mammary gland and eventual clearance of the organism in mmany cases; however, changes in immune function i.e. PMN diapedesis, ROS production, phagocytosis, cytokine production at different stages of lactation, and parity influence severity of clinical mastitis and outcome of IMI. Following parturition cows experience a state of immune dysfunction leading to uncontrolled inflammation and increased incidence and severity of infectious diseases (Fig 3). Increased nutritional demands of lactation in addition to dramatic changes in hormone profiles following calving are thought to regulate aspects of the immune response (Burvenich et al., 2007, Sordillo, 2016).

During pregnancy the maternal immune system is presented with the challenge of needing to concurrently tolerate the growth of a semi-allogenic fetus and respond to invading pathogens. Highly inflammatory, cell-mediated T helper 1 and T helper 17 type responses in pregnancy have been shown to be detrimental to the health of the fetus, thus, the maternal immune system must be carefully regulated. Though highly inflammatory responses appear to negatively impact pregnancy success, the original dogma that the maternal immune system is suppressed and biased towards Th2/Treg-type responses throughout pregnancy in order to tolerate the conceptus is an oversimplification of maternal immune tolerance. Maternal immune regulation in mammalian pregnancy is dynamic, and dairy cattle show varying responses to infectious agents a different stages of pregnancy (Quesnell et al., 2012, Sipka et al., 2013). Clinical signs of mastitis typically do not accompany infection within the dry period, not even IMI caused by *E. coli* which typically elicit highly inflammatory responses in lactation (Quesnell et al., 2012, Sipka et al., 2013,). This suggests the relationship between maternal immune regulation and immune response in the non-lactating mammary gland is complex.

There have been attempts to assist cows with their immune response through various products that can be administered during the dry period. Recently a commercially available bovine granulocyte colony stimulating factor preparation (pegbovigrastim) has been introduced to the dairy market globally. It is an immunomodulator of cytokines which has been shown to induce the production of mature neutrophils and stimulate their natural activity in the face of pathogenic challenge, especially in the post-partum period (Hassfurther et al., 2015). This same trial also showed a reduction in clinical mastitis. The commercially available systemic *E. coli* J5 mastitis vaccines are commonly administered in the non-lactating period of late gestation to aid in the reduction of clinical severity in postpartum *E. coli* mastitis through enhanced J5-specific IgM, IgG1, and IgG2 serum levels (Wilson et al., 2009).

Welfare

There has been large increase in the amount of milk produced over the entire lactation in dairy cows in the past decades. As reviewed and modeled by Zobel and coworkers (2015) a cow in 1975 might be dried off making about 10 kg (22 pounds) of milk whereas a cow in 2012 might be presented for abrupt dry-off making over 25 kg (55 pounds) of milk. These authors suggest that abrupt dry-off and thereafter acute involution might not be in the best interest of a cow's well-being under modern production circumstances as it might cause pain; however, there are also welfare considerations to reducing milk production prior to dry day such as hunger (if achieved by fed restriction) or frustration from not being milked (if achieved by decreasing

milking frequency). There has been some recent research investigating cabergoline as an aid to dry-off of modern dairy cows (Boutinaud et al. 2016, Bach et al. 2015). Cabergoline causes inhibition of prolactin secretion. In the trials mentioned it reduced prolactin secretion, udder engorgement, and milk leakage; however, a commercially available preparation is not currently marketed in the USA or Europe.

Antibiotic Therapy and Teat Sealants

Dry cow antibiotic therapy has been a part of dairy management for over a quarter of a century (Neave et al 1969). The many benefits of intramammary treatment at this time include: lower risk of residues in saleable food products, higher concentrations of longer-acting antibiotics can be used, and in the absence of milking antimicrobial activity can be maintained in the gland parenchyma for longer periods.

In the USA there are 6 FDA-approved drugs for intramammary use. Two recent on-farm clinical trials have shown non-inferiority among 4 of them (ceftiofur hydrochloride, cephapirin, and procaine penicillin G plus dihyrostreptomycin in one trial (Arruda et al., 2013a, Arruda et al., 2013b) and ceftiofur hydrochloride and cloxacillin in another (Johnson et al 2016)). In both trials all products had similar cure risks of greater than 85% and no difference between clinical mastitis risk, 305 ME milk production, or linear score. Despite an appealing cure risk it is reported that there is still a new infection risk from 13 to 25% (Godden et al 2003; Arruda et al 2013a). In a meta-analysis Halasa and coworkers (2009) reported that dry cow therapy is considered protective against *Streptococcus spp*., but that the existing evidence didn't support similar protection against coliforms or *Staphylococci spp*.

Both external and internal teat sealants have been shown to improve udder health. In one trial examining an external teat sealant those teats in which the sealant remained on for 3 days had the lowest linear score post-calving of all quarters in the trial including those that had antibiotic therapy only (Lim et al., 2007a). Unfortunately not all teats have adherence for 3 days with a range from 1 to 7 days (Lim et al., 2007b). Internal teat sealants, many of which are 65% bismuth subnitrate forming a physical barrier in the teat cistern and canal, have been shown to reduce the risk of dry period infection when compared to dry cow therapy alone (Godden et al., 2003; Rabiee and Lean, 2013). Moreover, in quarters without existing infections at dry-off internal teat seals were as effective as IM antibiotics at preventing new infections (Rabiee and Lean, 2013). Hygiene when applying internal teat sealants without antibiotics (and indeed hygiene is also important when administering IM antibiotics too!). Some dairy farms have had success implementing dry-off procedures on a trimming table instead of attempting to get these chores accomplished in the milking parlor.

Blanket dry cow therapy (BDCT), i.e. treating every quarter of every cow at dry-off, has been a mainstay of the National Mastitis Council's recommendations. It has certainly been useful in decreasing the prevalence of contagious mastitis associated with *Streptococcus agalactiae* and *Staphylococcus aureus*. Further it was likely in the best interest of a cow's well-being and a dairy farm's economic well-being to implement blanket dry cow therapy when the risks of an existing infection and/or the risk of a new IM infection were quite high at dry-off, especially when those risks were posed by non-coliforms and *Staphylococcus*. Fortunately since the early 1970s the risks of these infections has decreased markedly along with the prevalence of contagious mastitis

to the point where it might now be financially beneficial to some dairy producers to consider selective dry cow therapy (SDCT). This will also help address some of the public's concern regarding prudent antimicrobial use in dairy production.

In well managed herds 60-80% of quarters are likely not infected at dry-off and thus might not require antibiotic therapy, especially if a teat sealant is hygienically applied. Obviously this could reduce the cost and use of dry-off tubes by over 50%. Indeed in some Nordic (e.g. Sweden and Denmark) and other European (e.g. Netherlands) countries BDCT is no longer allowed. At present it is unlikely that every herd is ready for SDCT. Herds with no *Strep ag*., little to no *Staph aureus*, and BT SCC < 250,000 cells/mL could likely benefit from SCDT. It is less likely that many herds are ready to stop dry treating all together with the exception of a few cows although some elite herds certainly have done this with sustained success. For those herds employing SDCT one of the pivotal measures is distinguishing between cows that are likely to benefit from antibiotics and those that are not. An excellent trial preformed in Atlantic Canada demonstrated the success of on-farm culture using 3M Petrifilm (Cameron et al., 2014). A pilot study was performed in Minnesota using culture media designed for this purpose also showed great promise (Patel et al., 2016). In New York State we are evaluating an algorithm based on test day SCC and clinical mastitis events to identify cows that should or should not receive dry off antibitotics. It is likely that this indirect method is less sensitive at identifying infected cows than directly growing microorganisms. The algorithm data can be added to on-farm software to more easily make task lists on dry-off day. Thus far, at least in the well managed herds being tested, the low-risk cows not receiving antibiotics and only teat sealant are not worse off in the subsequent lactation.

Diet

There is an interaction between a cow's nutritional status and her immune function. Micronutrient management, especially the provision of adequeate vitamin E and selenium, has long been known to be important to immune function (Sordillo 2013, Politis 2012). Where it was once thought that providing more dietary metabolizable energy in the dry period might enhance general immune function by limiting negative energy balance, it is now been shown that not overfeeding dry cows has many metabolic benefits including controlling hyperketonemia (Mann et al 2015). Indeed controlled energy dry cow diets have been shown to have many benefits to the health and productivity of modern dairy cows. Moreover, it has been suggested that the frequently observed immunosuppression during spontaneous ketonemia during early lactation, and hence increased susceptibility to mastitis is most likely directly caused by elevated concentration of beta-hydroxybutyrate (BHB) (Zarrin et al 2014, Hillreiner et al 2016).

Summary

The dairy industry is under pressure to prudently use antibiotics will not compromising economic and cow well-being. Through a combination of available management and diagnostic interventions the selective use of antibiotics at the time of dry off represents a feasible opportunity. Dairies that have achieved good udder health may want consider working with their veterinarian to develop and implement a SDCT program after they have other important udder health considerations under control.

References

Arruda, A.G., S. Godden, P. Rapnicki, P. Gorden, L. Timms, S.S. Aly, T.W. Lehenbauer, and J. Champagne. 2013a. Randomized noninferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: I. Quarter-level outcomes. J. Dairy Sci. 96:4419-4435.

Arruda, A.G., S. Godden, P. Rapnicki, P. Gorden, L. Timms, S.S. Aly, T.W. Lehenbauer, and J. Champagne. 2013b. Randomized noninferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: II. Cow health and performance in early lactation. J. Dairy Sci. 96:6390-9.

Bach, A., A. De-Prado, and A. Aris. 2015 Short communication: The effects of cabergoline administration at dry-off of lactating cows on udder engorgement, milk leakages, and lying behavior. J. Dairy Sci. Oct. 98(10):7097-101.

Barkema, H.W., Y.H. Schukken, T.J. Lam, M.L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J. Dairy Sci. 81:411-9.

Boutinaud, M., N.Isaka, V. Lollivier, F. Dessauge, E. Gandemer, P. Lamberton, A.I. De Prado Taranilla, A. Deflandre, and L.M. Sordillo. 2016 Cabergoline inhibits prolactin secretion and accelerates involution in dairy cows after dry-off. J. Dairy Sci. Jul. 99(7):5707-18.

Burvenich, C., V. Van Merris, J. Mehrzad, A. Diez-Fraile, and L. Duchateau. 2003. Severity of *E. coli* mastitis is mainly determined by cow factors. Vet Res. 34:521-64.

Cameron, M., S.L. McKenna, K.A. MacDonald, I.R. Dohoo, J.P. Roy, and G.P. Keefe. 2014. Evaluation of selective dry cow treatment following on-farm culture: Risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J. Dairy Sci. 97:270-284.

Dingwell, R.T., K.E. Leslie, Y.H. Schukken, J.M. Sargeant, L.L. Timms, T.F. Duffield, G.P. Keefe, D.F. Kelton, K.D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev. Vet. Med. 63:75-89.

Godden, S., P. Rapnicki, S. Stewart, J. Fetrow, A. Johnson, R. Bey, and R. Farnsworth. 2003. Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. J. Dairy Sci. 86:3899-3911.

Green, M.J., L.E. Green, G.F. Medley, Y.H. Schukken, and A.J. Bradley. 2002. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. J. Dairy Sci, 85:2589-99.

Halasa, T., O. Østerås, H. Hogeveen, T. van Werven, and M. Nielen. 2009b. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. J. Dairy Sci. 92:3134-3149.

Hassfurther, R.L., T.N. TerHune, and P.C. Canning. 2015. Efficacy of polyethylene glycol-conjugated bovine granulocyte colony-stimulating factor for reducing the incidence of naturally occurring clinical mastitis in periparturient dairy cows and heifers. Am J Vet Res. Mar. 76(3):231-8.

Hillreiner, M., C. Flinspach, M.W. Pfaffl, and H. Kliem. 2016 Effect of the Ketone Body Beta-Hydroxybutyrate on the Innate Defense Capability of Primary Bovine Mammary Epithelial Cells. PLoS One. Jun 16:11(6).

Johnson, A.P., S.M. Godden, E. Royster, S. Zuidhof, B.Miller, and J. Sorg. 2016. Randomized noninferiority study evaluating the efficacy of 2 commercial dry cow mastitis formulations. J. Dairy Sci. Jan. 99(1):593-607.

Lim GH, Kelton DF, Leslie KE, Timms LL, Church C, Dingwell RT. 2007b. Herd management factors that affect duration and variation of adherence of an external teat sealant. J. Dairy Sci. Mar. 90(3):1301-9.

Lim, G.H., K.E. Leslie, D.F. Kelton, T.F. Duffield, L.L. Timms, and R.T. Dingwell. 2007a. Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period. J. Dairy Sci. Mar. 90(3):1289-300.

Mann, S., F.A. Leal Yepes, T.R. Overton, J.J. Wakshlag, A.L. Lock, C.M. Ryan, and D.V. Nydam. 2015. Dry period plane of energy: effects on feed intake, energy balance, milk production and composition in transition dairy cows. J. Dairy Sci. 98:3366-82.

Neave, F.K., F.H. Dodd, R.G. Kingwill, and D.R. Westgarth. 1969. Control of mastitis in the dairy herd by hygiene and management. J. Dairy Sci. 52:696-707.

Patel, K., S. Godden, E. Royster, J. Timmerman, B. Crooker, and N. McDonald. Pilot study: Evaluation of the effect of selective dry cow therapy on udder health. Proc. MN Dairy Health Conference. May 19-20, 2016. Bloomington, MN.

Politis, I. 2012. Reevaluation of vitamin E supplementation of dairy cows: bioavailability, animal health and milk quality. Animal. 2012 Sep. 6(9):1427-34.

Quesnell, R.R., S. Klaessig, J.L. Watts, and Y.H. Schukken. 2012. Bovine intramammary Escherichia coli challenge infections in late gestation demonstrate a dominant antiinflammatory immunological response. J. Dairy Sci. 95**:**117-26.

Rabiee, A.R., and I.J. Lean. 2013. The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. J. Dairy Sci. 96:6915-6931.

Sipka, A., A. Gurjar, S. Klaessig, G.E. Duhamel, A. Skidmore, J. Swinkels, P. Cox, and Y. Schukken. 2013. Prednisolone and cefapirin act synergistically in resolving experimental *Escherichia coli* mastitis. J. Dairy Sci. 96:4406-18.

Smith, K., and D. Todhunter. 1982. The physiology of mammary glands during the dry period and the relationship to infection. Pages 87 to 98 in Proceeding of the National Mastitis Council 21st Annual Meeting, Washington, DC. National Mastitis Council, Inc., Arlington, VA.

Sordillo, LM. 2013. Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Vet Med Int. 2013:154045.

Sordillo, L.M., and S.C. Nickerson. 1988. Morphometric changes in the bovine mammary gland during involution and lactogenesis. Am. J. Vet. Res. 49:1112–1120.

Sordillo, L.M. 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci, 99**,** 4967-82.

Wilson, D.J., B.A. Mallard, J.L. Burton, Y.H. Schukken, and Y.T. Grohn. 2009. Association of Escherichia coli J5-specific serum antibody responses with clinical mastitis outcome for J5 vaccinate and control dairy cattle. Clin Vaccine Immunol. 16:209-17.

Zarrin, M., O. Wellnitz, H.A. van Dorland, and R.M. Bruckmaier. 2014 Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. J. Dairy Sci. 97(1):330-9.

Zobel, G., D.M. Weary, K.E. Leslie, and M.A. von Keyserlingk. 2015. Invited review: Cessation of lactation: Effects on animal welfare. J. Dairy Sci. Dec. 98(12):8263-77.

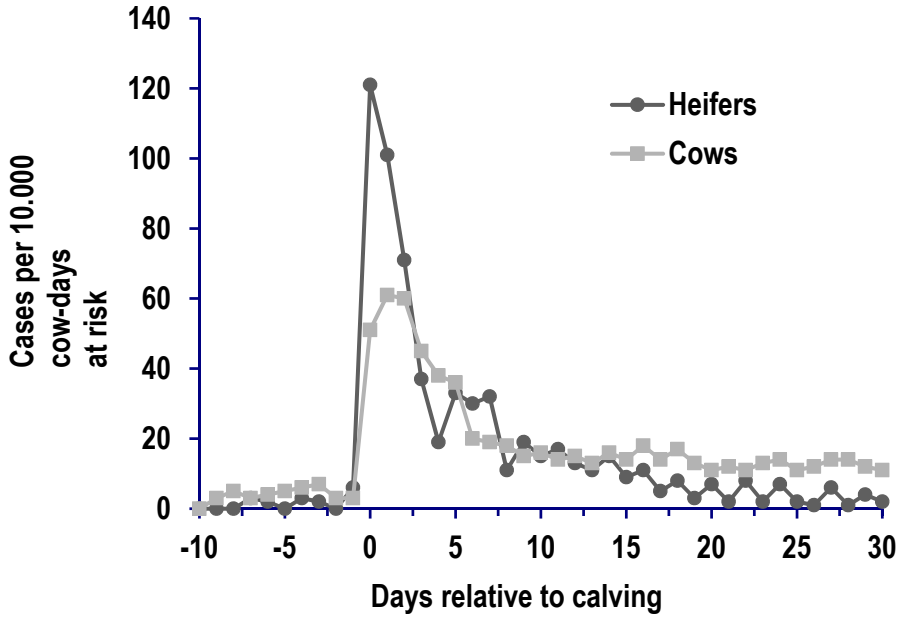

Figure 1. Distribution of incidence rate of clinical mastitis of weeks following calving. Adapted from Barkema et al. (1998).

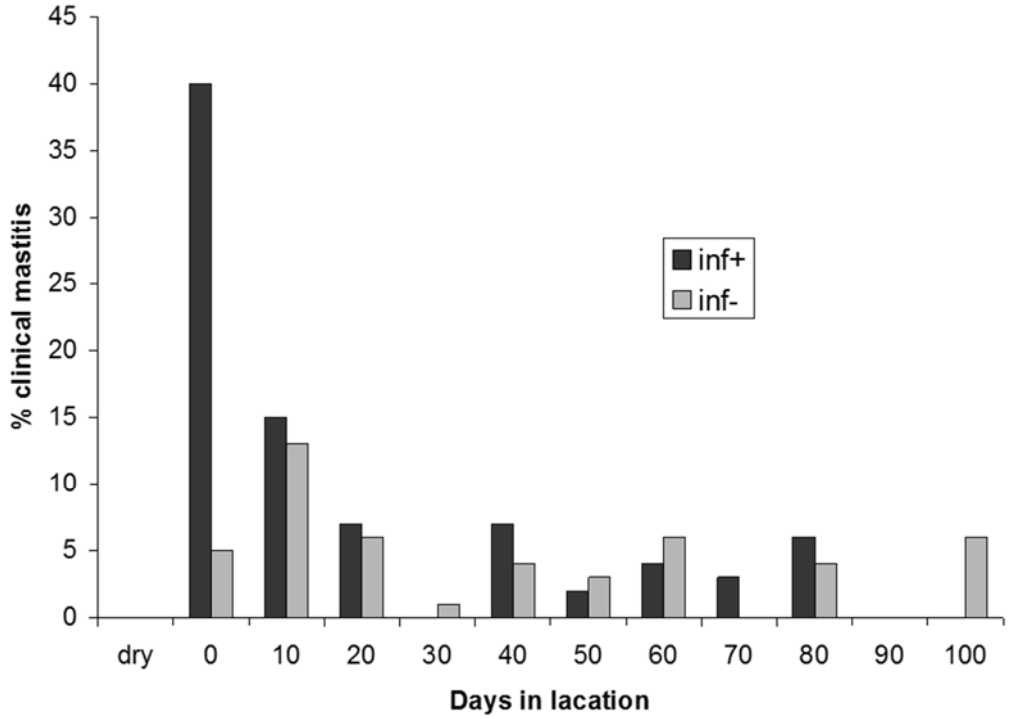

Figure 2. Clinical mastitis in early lactation. Solid black bars = Infection in dry period; Gray bars = No Infection in dry period. Adapted from Green et al. (2002).

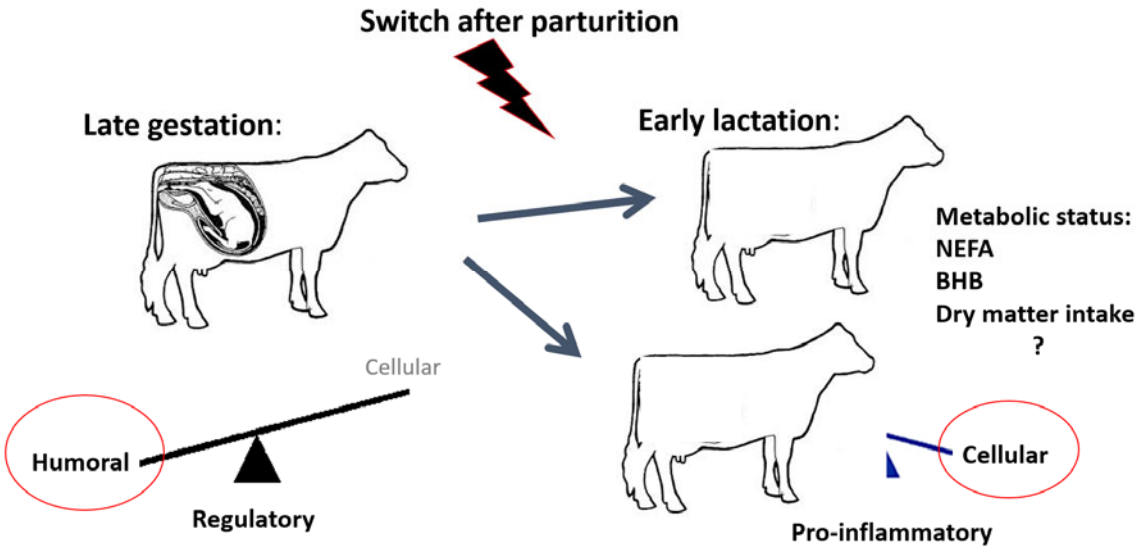

Figure 3. During gestation the maternal immune system is a state of relative tolerance. After parturition the immune system is transitioned to a pro-inflammatory state. The immune function can be influenced by dry period and early post-partum endocrine factors and energy balance. Adapted from Sipka, A. (2016).

Whole-genome Sequencing Analysis of Antimicrobial Resistance Genes in *Streptococcus uberis* and *Streptococcus dysgalactiae* isolates from Canadian Maritime Dairy Herds

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Introduction

Streptococcus uberis (*S. uberis*) and *Streptococcus dysgalactiae* (*S. dysgalactiae*) are classified as Gram positive, catalase-negative cocci (PNC) microorganisms and are frequently isolated in Canadian dairy farms (Olde Riekerink et al., 2008). Antimicrobial resistance (AMR) has been reported in different mastitis pathogens, to various degrees, in both short and long duration studies (Oliver and Murinda, 2012). The advent of whole genome sequencing (WGS) supports the molecular characterization and detection of multidrug resistance repertoires in mastitis pathogens, and allows comprehensive comparison to phenotypic characteristics.

Materials and Methods

S. uberis and *S. dysgalactiae* isolates were obtained from the Mastitis Pathogen Culture Collection (MPCC) of the Canadian Bovine Mastitis Research Network (CBMQRN) and were originally recovered from 18 commercial dairy herds located in three Maritime Canadian provinces (Reyher et al., 2011). Bacterial cultures and antimicrobial minimum inhibitory concentration (MIC) testing against eight antimicrobials: ampicillin, ceftiofur, cephalothin, erythromycin, penicillin, penicillin-novobiocin, pirlimycin, and tetracycline, were performed according to methodology reported by Cameron et al., (2016). WGS was performed at the Genome Sciences Centre (British Columbia) using the Illumina Genome Hi seq Analyzer. The sequences were aligned against reference genomes, and were annotated and compared with AMR databases using the tools available in the all-bacterial Bioinformatics Resource Center (patricbrc.org). Univariate descriptive and bivariate analyses (Fisher's exact test) were used to describe the frequency of AMR genes among genomes, the distribution of AMR and MIC values, and to determine the association between genotypic resistance and phenotypic susceptibility patterns. Moreover, a two-level logistic model (considering herd and isolate as random effects) was developed to assess the effect of genomic and epidemiologic variables with the phenotypic resistance as the outcome variable.

Results and Discussion

Seventy three isolates with complete WGS and MIC information, representing 18 dairy herds from the Maritime region of Canada, were included in the analysis and were part of previous phenotypic AMR study (Cameron et al., 2016). A total of 23 unique AMR genes were found in the bacterial genomes, with a mean number of 8.1 (minimum: 5; maximum: 13) per genome. Overall, there were 10 genes exclusively present in *S. uberis* genomes and two genes unique to the *S .dysgalactiae* genomes; 11 genes were common to both bacterial species. The two-way tabulations of phenotypic susceptibility (intermediate + resistant/susceptible; stratified by antimicrobial and species) and the absence/presence of AMR genes (stratified by class: beta-lactam, macrolide, lincosamide, and tetracycline) showed association between the presence of

linb ($P = 0.002$) and *lnub* ($P < 0.001$) genes and phenotypic resistance to lincosamides, and the presence of *tetM* ($P = 0.015$) and *tetS* ($P = 0.064$) genes and phenotypic resistance to tetracyclines in *S. uberis* isolates. The multivariate logistic model showed that the odds of resistance was 4.35 times higher when there were > 11 AMR ($P < 0.01$) genes present in the genome, compared with genomes with < 7 AMR genes. The log odds of resistance was lower for *S. dysgalactiae* than *S. uberis* ($P = 0.031$). When the within herd somatic cell count was > 250,000 cells/mL, a trend towards higher odds of resistance compared with the baseline category of <150,000 cell/mL was observed, however the coefficient and overall effect was non-significant ($P = 0.16$). When the isolate was recovered from a post-mastitis sample, the resistance was lower when compared with isolates recovered in non-clinical lactating quarters ($P = 0.01$).

Conclusions

The combination of phenotypic data (MIC) and WGS information is a novel approach for the field of udder health studies and provides new insight into the AMR potential of two major mastitis pathogens in Canadian dairy farms. Both bacterial species showed AMR, but was more common with *S. uberis* than *S. dysgalactiae* isolates, with association between phenotypic and genotypic characteristics. The likelihood of phenotypic AMR is determined by the number of AMR genes and epidemiological characteristics of the isolates

References

Cameron, M., M. Saab, L. Heider, J.T. McClure, J.C. Rodriguez-Lecompte, and J. Sanchez. 2016. Antimicrobial Susceptibility Patterns of Environmental Streptococci Recovered from Bovine Milk Samples in the Maritime Provinces of Canada. *Front. Vet. Sci.* 3:79.

Olde Riekerink, R.G.M., H.W. Barkema, D.F. Kelton, and D.T. Scholl. 2008. Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. *J. Dairy Sci.* 91:1366–1377.

Oliver, S.P., and S.E. Murinda. 2012. Antimicrobial Resistance of Mastitis Pathogens. *Vet. Clin. North Am. Food Anim. Pract.* 28:165–185.

Reyher, K.K., S. Dufour, H.W. Barkema, L. Des Côteaux, T.J. DeVries, I.R. Dohoo, G.P. Keefe, J.-P. Roy, and D.T. Scholl. 2011. The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *J. Dairy Sci.* 94:1616–1626.

Detection of Reservoirs of *Strep. agalactiae* in Automatic Milking Systems

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Introduction

Streptococcus agalactiae is a costly disease causing milk yield reductions and additional costs in dairy herds worldwide. From the beginning of 2000, the number of Danish dairy herds infected with *Strep. agalactiae* has increased after a period of low prevalence[1]. The re-emergence has renewed the focus on *Strep. agalactiae* management and control. The focus of this study was to examine possible reservoirs of *Strep. agalactiae* contamination on automatic milking unit (AMU) equipment.

Materials and Methods

The study was carried out in two AMS herds operating with Lely robots. All AMU units were tested and corrected by milk quality technicians. Eligible cows were chosen based on conductivity and PCR results on quarter/cow milk. A systematic sampling protocol was developed for the isolation of *Strep. agalactiae* from AMU surfaces exposed to udder, teats and milk during milking. Swab samples were collected before and after manual- and full automatic AMU cleaning as a baseline for the most contaminated or the cleanest environment a cow would meet at milking. Manual cleaning was carried out by the farmers and done with foam in herd 1, and chlorine water during the first visit and foam during the second visit in herd 2. After cleaning, 4 cows, the first infected with *Strep. agalactiae* followed by 3 uninfected cows, respectively, were milked subsequently. Swab samples (eSWAB^{TM}) were obtained from 10 AMU surfaces during and after each milking: from the brushes before disinfection, the brushes after disinfection, the brush motor, on top of and under the brush cap (if present), the mouthpieces and deep within the liners, the Lely Pura steam cleaning units and the laser-head. To evaluate possible carry-over in the milk recorder, milk samples were collected from the milk collection jar on the AMU after each cow. The entire procedure with manual- and full automatic cleaning, followed by milking 1 infected cow and 3 uninfected cows is referred to as "one sample sequence".

Samples were processed in the laboratory within 12 h. Selective enrichment and culture were done by incubation in Todd Hewitt broth with streptococcal selective supplement for 12 hours and plating on a Modified Edward's Medium (Oxoid) containing 2% washed sheep erythrocytes and 2% of a β-toxin producing *S. aureus* filtrate[2]. The Edward's plates were incubated for 48 hours and read after 24 ±4 hrs and 48 ±4 hrs. Typical streptococcal colonies with a visible CAMP reaction were selected and confirmed as *Strep. agalactiae* using latex agglutination test for Lancefield group-B (SLIDEX, Biomérieux Industry). Milk from the milk collection jar was cultured both directly and after selective enrichment and incubation with the Todd Hewitt broth.

Results

A total of 9 sample sequences from 6 AMUs, two in herd 1 and four in herd 2 were completed. A total of 460 swab samples were analyzed and *Strep. agalactiae* was isolated at least once from nine out of the ten surfaces investigated with a total of 104 positive samples (22.6%). AMU

surfaces with the highest proportion of positive samples were the liner mouthpieces (22.0%), brush-1 (20.0%), deep within the liners (18.0%), the laser-head (18.0%) and on top of the brush cap (16.7%). The contamination of *Strep. agalactiae* before the manual and full automatic AMU cleaning was7.5%. One third (33.8%) of all AMU surface samples were positive after an infected cow was milked. The number of positive samples isolated from the ten AMU surfaces decreased during the sample sequence indicating a declining level of *Strep. agalactiae* contamination of the AMU after each milking of an uninfected cow.

Strep. agalactiae carry-over in the milk recording jar was detected in 7 out of 9 sample sequences; in two cases *Strep. agalactiae* was isolated after milking of all three uninfected cows.

Discussion

The full automatic AMU cleaning cycle generally appears to remove *Strep. agalactiae* contamination efficiently from the liners, steam cleaning units and the brushes. The manual AMU cleaning with foam seemed to be more effective than using a bucket of chlorine water and a brush (both methods are recommended by Lely). The frequent isolation of *Strep. agalactiae* on AMU surfaces between milkings indicates that the automatic cleaning cycle between cows may not sufficiently eliminate *Strep. agalactiae* contamination from infected cows. However, steam cleaning of the interior AMU appeared superior to water. Brush disinfection between cows was repeatedly inadequate, as *Strep. agalactiae* in many instances was isolated. The developed sample sequence protocol identified possible sites of risk for transmission in the AMU during routine milking. The highest contamination of AMU surfaces with *Strep. agalactiae* was observed after milking an infected cow. Thus, uninfected cows being milked immediately after an infected cow may be at a higher risk of infection. However, *Strep. agalactiae* was, in some cases, found up to 3 milkings after the infected cow milking. Despite thorough maintenance and monitoring by quality technicians and the companies' technicians, inadequate function was observed. The type and frequency of inadequate function may differ within and between herds, with herd specific risks and modes of transmission. The carry-over observed in the milk recording jar indicates that samples taken for PCR analysis during milk recording in AMS may lead to false positive test results and may result in unnecessary treatment and culling of cows.

Conclusions

Viable *Strep. agalactiae* can be isolated frequently on AMU surfaces one to three milkings after the milking of an infected cow. The developed sampling sequence protocol is a useful tool to identify AMU specific sites of risk of transmission with *Strep. agalactiae* and to evaluate the efficiency of automatic and manual cleaning practices.

References

1. Katholm, J., 2010. *Streptococcus agalactiae* - An Increasing Problem in Scandinavia. The Nordic Dairy Association's Committee for Milk Quality, Mastitis symposium, Rebild, Denmark.

2. Jørgensen, H.J., A.B. Nordstoga, S. Sviland, R. Zadoks, L. Sølverød, B. Kvitle, and T. Mørk, 2016. *Streptococcus agalactiae* in the Environment of Bovine Dairy Herds – Rewriting the Textbooks. Vet Mic. 184, pp.64–72.

Costs of Mastitis and Milk Quality Management Practices Perceived by Dairy Producers in the Southeastern United States

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Introduction

Mastitis is the costliest disease to the dairy industry worldwide. Producers take many different steps to manage milk quality on their farms. Pre and post-milking teat disinfection, coliform mastitis vaccinations, and antibiotic treatment have all proved beneficial practices to control milk quality. The three of objectives of this study were (1) to determine how much southeastern U.S. dairy producers spend on milk quality control practices, (2) to determine if the herd bulk tank somatic cell score (SCS) had an effect on mastitis management costs and (3) to quantify producer perceptions of mastitis costs.

Material and Methods

Data Collection

Researchers from four universities in the southeastern United States completed 175-question surveys on 282 farms in TN (n = 83), KY (n = 96), VA (n = 96), and MS (n = 7) from June 22, 2014 to June 21, 2015 as a part of the Southeast Quality Milk Initiative project. Herd size ranged from 32 to 2,500 lactating cows with a mean (± SD) of 228 ± 329 and a mean 2014 bulk tank SCS of 7.71 ± 0.58. To account for a distribution of bulk tank SCS, each state's SCS distribution was broken into thirds and averaged together into high, medium, and low herds. Survey answers either consisted of producer cost estimates or provided information allowing researchers to calculate the costs spent on management practices. The management practices of interest included pre and post-milking teat disinfectant costs, coliform mastitis vaccination costs, and intramammary antibiotic therapy costs.

Data Analysis

Using the GLM procedure in SAS 9.4 (SAS Inc., Carry, NC), a univariate analysis was completed to determine variables of significance ($P < 0.05$) to be included in a final multivariate analysis. Non-significant variables in the multivariate model were removed one-by-one using stepwise backward elimination until all remaining variables were significant or a part of a significant interaction. This process was repeated for each of the six variables of interest.

Results

Mean (± SE) management costs were \$0.04 ± \$0.03/cow/d, \$0.04 ± \$0.04/cow/d, \$2.65 ± \$1.18/vaccination protocol, and \$15.08 ± \$8.81/mastitis case, for pre and post-milking teat disinfectant, coliform mastitis vaccination, and intramammary antibiotic treatment, respectively.

Intramammary antibiotic cost was the only variable of interest that bulk tank SCS significantly ($P = 0.03$) influenced. With every one-point increase in bulk tank SCS, costs spent on antibiotics increased by $1.07. With every one-point increase in SCS producers were using approximately an additional half of an antibiotic tube per mastitis case. Producers that had made management changes in the past year to deal with a higher bulk tank SCS than their goal spent significantly ($P = 0.02$) more on intramammary antibiotics than those who did not make management changes.

Researchers also wanted to determine if dairy producers had an understanding of how both clinical and subclinical mastitis affected their business. In order to determine this, producers were asked to provide an estimate of how much a case of mastitis costs them. Estimates were $269.77 ± $380.62 and $240.77 ± $304.43 and ranged from $0.00 to $3,000.00 and $2,000.00 for clinical and subclinical mastitis, respectively. Bulk tank SCS did not significantly ($P = 0.12$) influence the producer's estimate of the cost of clinical mastitis. However, bulk tank SCS did significantly ($P = 0.001$) affect their estimates of the cost of subclinical mastitis. For every one-point increase in bulk tank SCS, producer estimates increased by $4.01.

Conclusions

Bulk tank SCS had an effect only on the cost of intramammary antibiotics. This may suggest that dairy producers in the southeast United States were using antibiotics to manage milk quality in their operations. When asked for an estimate of clinical and subclinical mastitis, dairy producer estimates were close to those provided through economic analysis. However, the variability in estimates was large with the most common answer being no answer at all. The relationship between bulk tank SCS may suggest that dairy producers with a high SCS might be more aware of the economic benefit they are missing from producing lower quality milk.

Acknowledgments

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Persistence of Coagulase Negative Staphylococcal Intramammary Infection in Dairy Goats

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Introduction

Coagulase negative staphylococci (CNS) are the most prevalent bacteria isolated from intramammary infections (IMI) of dairy goats. More than 15 different species have been associated with IMI. The relationship between CNS IMI, milk somatic cell count (SCC) and milk loss is less clear in goats than in cows (Koop et al., 2010). The conflicting reports regarding the importance of CNS IMI in goats may be explained by the variation between studies with regard to distribution of CNS species, varying pathogenicity between species, the presence of confounding factors affecting outcome measures, and differences in methods of speciation. The objective of this study was to describe the persistence of species-specific CNS IMI in goats from a large dairy goat herd in Missouri.

Materials and Methods

Udder-half level milk samples were aseptically collected from all 909 lactating goats for bacterial culture and milk SCC enumeration. Bacterial cultures were performed according to NMC guidelines, and cultures yielding 100 CFU/ml CNS were used to define an IMI. Goats with at least one udder-half yielding culture growth of a single colony morphology of CNS were enrolled for two additional udder-half level milk samplings at 1-monthly intervals and samples were cultured as described above. All CNS isolates were stored in phosphate buffered glycerol at -80^{o}C for further characterization. Isolates were identified to the species-level using matrix assisted laser desorption/ionization time of flight (MALDI-ToF) mass spectrometry, which based on preliminary data had an excellent agreement with gene sequencing. All isolates were run in duplicate, and a score $\geq$ 2.0 on at least one of the duplicates was considered adequate for species identification. Isolates that did not meet the identification criteria were subjected to PCR amplification and sequencing of the *tuf* gene. Intramammary infection status was defined based on the presence of the same species of CNS in 1, 2, or 3 of the samples from each udder half (Table 1).

Results

Results of the initial sampling revealed 253 udder-halves with a CNS IMI on 220 of the 909 goats. Of these 253 udder-half IMI, 53 were excluded due to missing data at 1 or more of the samplings (e.g., missing or contaminated sample). An additional 24 halves were excluded due to unsuccessful identification of at least one of the CNS isolates from the 3 samplings. From the 176 IMI included in the final analysis, 472 CNS isolates were characterized to the species-level. Of these, 458 isolates were identified using MALDI-ToF and 14 isolates were identified by *tuf* gene sequence. Overall, 12 different CNS species were identified. Following speciation, IMI were classified based on the number of samples positive for the same CNS species (Table 1). Note that 7 IMI had a different CNS species identified after the first sampling.

Table 1. Species-specific persistence over 3 consecutive monthly samplings by CNS species.

	Number of infected halves by culture results				
Species	CNS-0-0	CNS-CNS-0	CNS-0-CNS	CNS-CNS-CNS	Total
S. simulans	6	1	1	63	71
S. xylosus	10	0	0	24	34
S. caprae	2	0	0	16	18
S. epidermidis	3	0	0	11	14
S. chromogenes	0	0	0	10	10
S. arlettae	10	0	0	0	10
S. lentus	6	0	0	0	6
S. equorum	2	0	0	0	2
S. capitis	1	0	0	0	1
S. cohnii	0	0	0	1	1
S. haemolyticus	1	0	0	0	1
S. hominis	1	0	0	0	1
Total	42	1	1	125	169

Discussion

The most commonly identified species were *S. simulans*, *S. xylosus*, *S. caprae*, and *S. epidermidis*. Most IMI with these species persisted for the 3-month period. *S. xylosus*, *S. caprae*, and *S. epidermidis* have been previously associated with persistence of IMI in goats (Contreras et al., 1997; Moroni et al., 2005; Koop et al., 2012), but not *S. simulans*. Future work will include subspecies classification using pulsed-field gel electrophoresis to truly define whether IMI persist over time. Additionally the correlation between IMI and SCC will be evaluated.
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References

Contreras, A., J.C. Corrales, A. Sanchez, and D. Sierra. 1997. Persistence of subclinical intramammary pathogens in goats throughout lactation. J. Dairy Sci. 80(11):2815-9.

Koop, G., S. De Vliegher, A. De Visscher, K. Supré, F. Haesebrouck, M. Nielen, and T. van Werven. 2012. Differences between coagulase-negative *Staphylococcus* species in persistence and in effect on somatic cell count and milk yield in dairy goats. J. Dairy Sci. 95(9):5075-84.

Koop, G., T. van Werven, H.J. Schuiling, and M. Nielen. 2010. The effect of subclinical mastitis on milk yield in dairy goats. J. Dairy Sci. 93(12):5809-17.

Moroni, P., G. Pisoni, M. Antonini, G. Ruffo, S. Carli, G. Varisco, and P. Boettcher. 2005. Subclinical mastitis and antimicrobial susceptibility of *Staphylococcus caprae* and *Staphylococcus epidermidis* isolated from two Italian goat herds. J. Dairy Sci. 88(5):1694-704.

Teat Recovery after Machine Milking as Determined by Ultrasonography and its Association with Teat-end Shape

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Introduction

Intramammary infections from bacteria entering the gland via the teat canal are the most frequent cause of mastitis. The impaired ability of the teat to act as a barrier is a major risk factor for bacterial invasion (Mein, 2012). There is consensus that machine milking induces changes in teat tissues which are differentiated into short-term and long-term changes.

Long-term changes are the adaptation of teat tissue to machine milking over weeks and include teat-end callosity, thickness, and roughness (Neijenhuis et al., 2000). Short-term changes are defined as tissue responses to a single milking. There is agreement that short-term changes inhibit timely closure of the teat canal orifice after milking and therefore also increase the risk of intramammary infections by mastitis pathogens (Neijenhuis et al., 2001). While long-term changes in teat condition have been shown to be associated with teat characteristics such as teat-end shape (Neijenhuis et al., 2000), the association between short-term changes and teat characteristics has not been established. The objective of our study was to investigate the association of machine milking induced short-term changes in teat condition as measured by ultrasonography with teat-end shape.

Materials and Methods

Holstein cows (n=125) were enrolled in a prospective cohort study. Machine milking induced short-term changes of the left front and the right hind teat were assessed with ultrasonography before milking (t-1), immediately (t0) and 1, 3, 5, and 7 hours after milking (t1 – t7). Teat parameters assessed were teat canal length (TCL), teat-end diameter at the midpoint between the distal and the proximal end of the teat canal (TMD), and relative change (%) of the teat wall thickness (TWR). Teat-end shape was assessed visually and classified into three categories: pointed, flat, and round.

Analytical Approach

To study the association between measured teat parameters and teat-end shape (pointed, flat, and round) over time, a general linear mixed model was used. Kramer´s posthoc test was used to control for experiment-wise error rate for comparison of means across different teat characteristics and time points. Recovery time of teat parameters was assessed by comparison of postmilking (t0 – t7) with premilking (t-1) values.

Results

Teat canal length was associated with teat-end shape ($P < 0.0001$) and time of measurement ($P < 0.0001$). There was an interaction between teat-end shape and time ($P < 0.0001$). Average (LSM ± SE) TCL at t-1 was 14.9 ± 0.3, 12.3 ± 0.3, and 13.8 ± 0.2 mm in teats with pointed, flat, and

round teat-end shape, respectively. Immediately after milking (t0) average TCL increased to 15.6 ± 0.3, 13.2 ± 0.3, and 14.9 ± 0.2 mm, in teats with pointed, flat, and round teat-end shape, respectively. Teat recovery took one hour in pointed and flat teats, while the pre-milking value was not reached within the seven hours in teats with round teat-end shape.

Teat-end diameter at the midpoint between the distal and the proximal end of the teat canal was associated with teat-end shape ($P < 0.0001$) and time of measurement ($P < 0.0001$). There was an interaction between teat-end shape and time ($P < 0.0001$). Average (LSM ± SE) TMD at t-1 was 17.8 ± 0.2, 20.3 ± 0.2, and 18.4 ± 0.2 mm and increased to 18.1 ± 0.2, 20.5 ± 0.2, and 18.8 ± 0.2 mm at t0, in teats with pointed, flat, and round teat-end shape, respectively. Average TMD did not reach the original value (t-1) within the seven hours of ultrasound scanning.

Relative change in teat wall thickness was associated with teat-end shape ($P < 0.0001$) and time of measurement ($P < 0.0001$). Average (LSM ± SE) TWR increased to 115 ± 3, 114 ± 3, and 120 ± 2 % immediately after milking (t0) and reached the value before unit attachment (t-1) within three, three, and five hours after milking in teats with pointed, flat, and round teat-end shape, respectively. There were differences in TWR at t0 between teats with pointed and round teat-end shape ($P = 0.007$) and flat and round teats ($P = 0.002$), whereas no differences could be detected between teats with pointed and flat teat-end shape ($P = 0.7$).

Conclusion

The magnitude of machine milking induced short-term changes in teat condition were associated with teat-end shape. Recovery time of teats after machine milking was different in teats with different teat-end shape. Consideration of teat-end shape in milking liner design and milking machine settings has the potential to decrease the degree of short-term changes and potentially improve udder health.

References

Mein, G.A. 2012. The Role of the Milking Machine in Mastitis Control. Veterinary Clinics of North America: Food Animal Practice. 28:307-320.

Neijenhuis, F., H.W. Barkema, H. Hogeveen, and J.P. Noordhuizen. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. J. Dairy Sci. 83:2795-2804.

Neijenhuis, F., G.H. Klungel, and H. Hogeveen. 2001. Recovery of cow teats after milking as determined by ultrasonographic scanning. J. Dairy Sci. 84:2599-2606.

Udder Health without Dry Cow Antibiotics: The Potential of Shortening or Omitting the Dry Period

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Introduction

Preventive use of dry cow antibiotics is not allowed in several European countries among which the Netherlands. Development of a management system without use of preventive dry cow antibiotics justifies re-evaluation of other management measures with respect to udder health, such as reducing the length of the dry period (DP). Shortening or omitting the DP has been hypothesized to affect udder health in dairy cows, although reported effects are ambiguous (Van Hoeij et al., 2016). Other factors that improved energy balance (EB) such as dietary energy level, may improve udder health. Additionally, to our knowledge, all studies on the effect of dry period length (DPL) on udder health were carried out using cows that were treated with dry cow antibiotics at drying off.

The aim of this study was to compare the effect of an omitted or shortened DP without use of dry cow antibiotics on intramammary infections (IMI) and mastitis pathogens in milk at drying off and after calving, and on milk yield, somatic cell count (SCC), elevation of SCC, and clinical mastitis in subsequent lactation.

Materials and Methods

Holstein-Friesian cows (n = 130) were assigned randomly to 3 treatments: a 30-d DP fed a standard (STD) dietary energy level postpartum, with 8.5 kg/d concentrate, required for their expected milk yield (30-d DP (STD)) (n = 44); a 0-d DP fed a ration with the same STD dietary energy level as cows with a 30-d DP (0-d DP(STD)) (n = 44); or a 0-d DP fed a LOW concentrate level, with 6.7 kg/d concentrate, based on the expected milk yield of cows with a 0-d DP (0-d DP(LOW)) (n = 42). Experimental concentrate was provided individually over 6 periods per day by a computerized feeder located in the free-stall, separately from the basal ration. The basal ration was provided ad libitum and was based on grass silage and corn silage (6.4 MJ net energy for lactation / kg dry matter (DM)). Dry matter intake was measured and EB was calculated between 5 weeks prepartum and 44 weeks postpartum.

Milk yield was recorded daily and a composite sample of 4 milkings was analyzed for milk composition (fat, protein, lactose, and SCC) weekly. Quarter milk samples for bacterial culturing were collected at 5 weeks prepartum, 1 week postpartum and 5 weeks postpartum.
Postpartum indicators for udder health were SCC, at least 1 elevation of SCC between 3 and 44 weeks postpartum, and at least 1 case of clinical mastitis. An elevation of SCC was defined as SCC ≥200,000 cells/mL after two weeks with SCC<200,000 cells/mL.

Results

Total lactation yield and average daily fat-and-protein-corrected milk (FPCM) yield were 30% and 18% greater in cows with a 30-d DP than in cows with a 0-d DP. Average EB in week 1 to 5

postpartum, SCC, and occurrence of at least 1 case of clinical mastitis were 3.3 times, 1.1 times, and 3 times lower in cows with a 30-d DP than a 0-d DP. Cows with a 0-d DP(LOW) or 30-d DP(STD) had a lower SCC when expressed as total output in milk, than cows with a 0-d DP(STD). The time from calving to first case of clinical mastitis was greater for cows with a 30-d DP(STD) than for cows with a 0-d DP(STD). The occurrence of IMI across the DP was not different between DP lengths. The prevalence of quarter milk samples without bacterial growth or with major pathogens or minor pathogens was not different among prepartum DP length (0-d DP or 30-d DP) or among treatments (0-d DP(LOW), 0-d DP(STD), 30-d DP(STD)). Furthermore, postpartum SCC was associated with parity, calving season, incidence of at least one case of clinical mastitis, FPCM yield, and lactation week. At least 1 elevation of SCC in the postpartum period was associated with parity and the average EB for the complete lactation. At least 1 case of clinical mastitis in the postpartum period was associated with average FPCM yield for lactation.

Discussion

Lower milk yield in cows with a 0-d DP could be related with a lower dilution of amount of somatic cells in milk and consequently a greater measured SCC, compared with cows with a 30-d DP (Steeneveld et al., 2013). Yield of FPCM is negatively related with SCC, irrespective of the presence of an IMI (Green et al., 2006). In the current study, correction of SCC for dilution in FPCM yield resulted in disappearance of the effect of DPL on SCC. This suggests that the difference in SCC between cows with a 0-d DP or 30-d DP mostly results from dilution, rather than from bacterial infection or proliferation rate, and that omitting the DP does not result in impaired udder health, compared with a 30-d DP.

Conclusion

Cows with a 0-d DP had a greater SCC postpartum than cows with a 30-d DP. After correction for differences in milk yield, differences in SCC among DPLs disappeared. Additionally, the occurrence of at least one elevation of SCC and the proportion of healthy cows across the DP were not different among DPs, and DPL was not associated with at least 1 elevation of SCC or case of clinical mastitis. The DP can, therefore, be omitted in cows, without detrimental effects on udder health in the subsequent lactation.

References

Green, L.E., Y.H. Schukken, and M.J. Green. 2006. On distinguishing cause and consequence: Do high somatic cell counts lead to lower milk yield or does high milk yield lead to lower somatic cell count? Prev. Vet. Med. 76:74-89.

Steeneveld, W., Y.H. Schukken, A.T.M. van Knegsel, and H. Hogeveen. 2013. Effect of different dry period lengths on milk production and somatic cell count in subsequent lactations in commercial Dutch dairy herds. J. Dairy Sci. 96:2988-3001.

Van Hoeij, R.J., T.J. Lam, D.B. De Koning, W. Steeneveld, B. Kemp, and A.T.M. Van Knegsel. 2016. Cow characteristics and their association with udder health after different dry period lengths. J. Dairy Sci.

Intention of Farmers toward Controlling Mastitis in Urban and Peri-urban Dairy Production Systems of Northwest Ethiopia

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In Ethiopia, dairy farmers get advice by the livestock development and veterinary services to improve udder health; however, farmers practice mastitis control measures infrequently. Understanding dairy farmers' intention may be helpful to design intervention strategies and enhance farmers' motivation (Valeeva et al., 2007). Therefore, the present study was conducted to identify determinants of dairy farmers' motivation toward controlling mastitis, and socio-demographic characteristics influencing dairy farmers' attitude, subjective norms (SN) and perceived behavioural control (PBC) in urban and peri-urban dairy production systems of Northwest Ethiopia.

Materials and Methods

Data Collection and Statistical Analysis

Questionnaires were administered in person to 134 randomly selected dairy farmers who kept Holstein Friesian x Zebu cross breed dairy cows in two regions in North-Western Ethiopia: Gondar and Bahir Dar. Using seven point Likert scale questions, farmers were interviewed about their attitude, SN, PBC and intentions of five mastitis control measures (MCM). The score for belief statements was multiplied by the corresponding outcome evaluation (OE), motivation to comply (MC), or perceived power of control (PPC) to obtain a product score. Cronbach's alpha was calculated on the product scores. Based on the product, each of the predictor variables (attitude, SN and PBC) were classified into three categories like no attitude, low attitude, or strong attitude. When respondents <5, the strong and the low categories summed together. Analysis was done by multivariable logistic regression modelling according to the framework of the Theory of Planned Behaviour.

Results

A positive intention to implement MCM was seen in the majority of the farmers: to implement any MCM (93%), to improve udder cleaning (87%), to improve stall hygiene (78%), to improve feeding of cows (76%), and to implement foremilk stripping (74%). A large number of dairy farmers had a positive attitude, positive SN and positive PBC to implement mastitis control. All respondents had positive attitude to implement any MCM. However, the number of farmers with a positive intention decreased when it comes to specific mastitis control measures (SMCM). Dairy farmers who had strong or low positive attitude had higher odds of intention to implement any MCM and foremilk stripping, and to improve stall hygiene compared to farmers who had low or no positive attitude (Table 1).

Of the background factors, experience of dairy farming and experience of mastitis during the last year influenced their attitude while gender and education level influenced SN.

Table 1. Final models describing the association between dairy farmers' attitude or subjective norms with their intention toward implementing any mastitis control measure, improving udder cleaning, stall hygiene and implementing foremilk stripping in Northwest Ethiopia.

Mastitis control measures	Explanatory variables	Level	OR[a]	95% CI[b]
Any mastitis control measure	A (BB)	Low	Ref.[c]	
		High	27.98	4.89-160.23
Improving udder cleaning	SN (NB * MC)	No	Ref.	
		Low	4.45	1.22-16.19
		Strong	4.61	0.39-54.37
Improving stall hygiene	A (BB * OE)	No	Ref.	
		Low	2.70	1.14-6.40
		Strong	2.26	0.21-24.49
Foremilk stripping	A (BB * OE)	No	Ref.	
		Low	3.97	1.18-13.37
		Strong	8.97	2.65-30.37
	SN (NB * MC)	No	Ref.	
		Low	4.87	1.79-13.26
		Strong	4.61	0.45-47.35

[a]Odds ratio, [b]95% confidence interval, [c]Reference category, A = Attitude, BB = behavioural beliefs, SN = subjective norm, NB = normative belief, MC = motivation to comply, OE = outcome evaluation.

More farmers intended to practice any MCM than farmers intended to implement SMCM. This could be due to lack of understanding the SMCM lack of evidence of effectiveness (Gunn et al., 2008) and due to the fact that implementation of SMCM is generally more difficult. A strongly positive SN was not significantly associated with intention while low positive SN were; this may be due to satisfaction of dairy farmers with the current mastitis situation even though farmers feel social pressure. Dairy farmers' PBC was not associated with any of the intentions of potential MCM. It implies that even though many farmers had the intention to control mastitis, they did not perceive control. In conclusion, in a situation where one does not perceive control, it is unlikely that a person will change behaviour. Therefore, to increase dairy farmers' intentions toward mastitis control in Northwest Ethiopia by increasing their PBC, interventions focusing on utilization of time and labour, and practical training on SMCM might be important.

References

Gunn, J.G., C. Heffernan, M. Hall, A. McLeod, and M. Hovi. 2008. Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. Preventive Veterinary Medicine. 84:310–323.

Valeeva, N.I., T.J.G.M. Lam, and H. Hogeveen. 2007. Motivation of dairy farmers to improve mastitis management. Journal of Dairy Science. 90:4466-4477.

Pilot Study: Impact of Using a Culture-guided Selective Dry Cow Therapy Program Targeting Quarter-level Treatment on Udder Health and Antibiotic Use

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Selective dry cow therapy (SDCT) is an approach whereby the decision to administer antibiotic (Ab) treatment at the end of lactation is based on knowing the likely infection status of the cow or quarter. Selective dry cow therapy programs have the potential to significantly reduce Ab use at dry-off (DO). However, early studies reported that SDCT programs were not as effective as blanket DCT (BDCT) programs, in that cows assigned to a SDCT program experienced worse udder health in the subsequent lactation as compared to cows assigned to a BDCT program (Berry and Hillerton., 2002; Scherpenzeel et al., 2014). Two limiting factors to previous SDCT programs may have been the use of less sensitive diagnostic tests at dry-off (e.g. SCC history) to identify cows with intramammary infection (IMI), as well as failure to use an internal teat sealant (ITS) to protect untreated quarters from IMI during the dry period. However, a more recent study that used a rapid culture system to target cow-level treatment decisions, and which used an ITS, reported equal udder health between the SDCT and BDCT programs (Cameron et al., 2014). The aim of this research was to demonstrate proof of concept in terms of safety and efficacy of adopting a culture-guided SDCT program that targeted diagnosis and treatment of individual quarters at the time of DO. We hypothesized that using a culture-guided SDCT program to guide quarter-level treatment decisions would be equally successful to BDCT in terms of promoting udder health while significantly reducing Ab usage.

Materials and Methods

The study was conducted at the University of Minnesota, Saint Paul campus dairy barn between July 2015 and March 2016. A total of 56 cows were enrolled two days prior to DO and randomly assigned to the two treatment groups: SDCT or BDCT. Aseptic quarter milk samples were collected 2 days prior to DO and again at 0-7 days in milk (DIM), and submitted for routine bacteriological culture for pathogen identification according to NMC guidelines. The quarter milk samples collected from the SDCT group prior to DO were also plated onto a new media developed as a part of Minnesota Easy™ culture system (University of Minnesota. St. Paul, MN) and incubated at 37 °C for 36 hr. On the day of DO, results of the 4Sight plate were read and reported as bacterial growth (G) or no growth (NG). For cows enrolled into the SDCT group, quarters that were positive for G were infused with Ab followed by an ITS at DO, while quarters with NG were infused solely with the ITS. All quarters of cows enrolled into the BCDT group were infused with Ab followed by the ITS at DO. Following administration of dry treatment, all quarters of all cows were dipped with a post-milking teat disinfectant. All clinical mastitis events were recorded by herd staff between DO and 30 days post-calving. Statistical analysis was conducted in SAS 9.4 (SAS Institute Inc., Cary, NC) using multivariable logistic regression models to evaluate the effect of treatment on risk for i) presence of IMI at calving, ii) cure and iii) new IMI, with cow included as random effect in the model to account for clustering of

quarters within cows. Additional explanatory variables considered for inclusion in the models included cow parity, previous lactation SCC, previous lactation total milk yield (kg) and dry period length (d). Significance was set at $P < 0.05$.

Results

Results showed no effect of treatment on quarter health measures after calving (risk for presence of IMI at calving, cure, new IMI) (Table 1). Antibiotics were used in 52% and 100% of quarters in the SDCT and BDCT groups, respectively.

Table1. Effect of using a culture-guided quarter-level SDCT program on udder health

Outcome parameters for model	Treatment group	Crude proportion affected	Odds Ratio (95% CI)	P – Value (Odds Ratio)
Risk for IMI at DO	SDCT	36.9%	1.228 (0.664, 2.293)	0.51
	BDCT	32.6%	Referent	
Risk for IMI at calving	SDCT	42.2%	1.120 (0.612, 2.050)	0.71
	BDCT	39.6%	Referent	
Risk for new IMI at calving	SDCT	40.2%	0.910 (0.484, 1.709)	0.76
	BDCT	41.5%	Referent	
Risk for cure	SDCT	82.3%	0.604 (0.119, 3.060)	0.53
	BDCT	88.0%	Referent	

Conclusions and Next Steps

Application of a culture-guided SDCT program targeting quarter-level diagnosis and treatment decisions resulted in equal udder health after calving, as compared to a BDCT program, while reducing Ab use by 48%. The results of this pilot study indicate that SDCT programs can be successfully applied at the quarter level. A next step will be to verify that this program can work in multiple commercial herds under different management conditions.

References

Berry, E.A., and J.E. Hillerton. 2002. The effect of selective dry cow treatment on new intramammary infections. J. Dairy Sci. 85:112–121.

Cameron, M., S.L. McKenna, K.A. Macdonald, I.R. Dohoo, J.P. Roy, and G.P. Keefe. 2014. Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J. Dairy Sci. 97:270-284.

Scherpenzeel, C.G.M., I.E.M. den Uijl, G. van Schaik, R.G.M. Olde Riekerink, J.M. Keurentjes, and T.J.G.M. Lam. 2014. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. J. Dairy Sci. 97:3606–3614.

The Antimicrobial Effects of Bovine Mammary Stem Cell Secretome on the Mastitis Pathogen *Klebsiella Pneumonia*

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Introduction

Klebsiella pneumonia mastitis is prevalent and frequently characterized by massive inflammation and widespread tissue necrosis (Schukken et al., 2012). An important limitation of intramammary antibiotics for the treatment of *Klebsiella* mastitis, aside from failed efficacy for certain formulations, is their inability to fully revert mastitis-induced structural damage in the udder to healthy tissue capable of full milk production. This makes the search for alternative treatment regiments that can simultaneously fight bacteria and restore tissue damage highly attractive. Bovine mammary stem/progenitor cells (BMaSC) drive the various developmental changes during pregnancy, lactation, and involution and their use as a potential stem cell therapy in the management of post-mastitis inflammatory damage has been proposed (Sharma and Jeong, 2013). Their antimicrobial properties in relation to mastitis have never been explored. Our objectives in this trial were to (i) describe the antimicrobial capacities of BMaSC conditioned media on *K. pneumonia*, (ii) determine whether manipulation of BMaSC can impact their antimicrobial function and to (iii) quantify the presence of antimicrobial peptides (AMPs).

Materials and Methods

Mammary tissue was harvested from the glands of cattle at slaughter. BMaSC were isolated by collagenase treatment of the tissue and further enriched using ultra-low attachment plates to allow for the formation of mammospheres. Primary mammary fibroblasts (BMF) were used as comparison cells. Aliquots of BMaSC or BMF were propagated, resuspended in antibiotic-free medium, and permitted to grow for 24h. After centrifugation, conditioned medium (CM) harvested from fibroblasts and stem cells was used for experiments. To determine the effect of CM on bacteria, mastitis-causing field strains of *K. pneumonia* were subjected to serial (1:2) dilutions of CM. A 10 µL bacterial suspension (100CFUs) was added to 90 µL of CM in a 96-well plate and incubated at 37°C for 16h. A positive control of incubated acellular media plus bacteria was included on each plate. Antibacterial activity of CM was measured using spectrophotometric readings (OD at 600nm) to quantify bacterial growth. To determine the antimicrobial effects of CM collected from manipulated BMaSC or BMF, pretreatments were first performed by seeding BMaSC or BMF in media supplemented with the immunostimulants LPS, α-toxin, IL17a, or heat-inactivated *S. aureus,* and experiments were performed as described above. In addition to describing alterations in bacterial growth, we also evaluated changes in the permeability of bacterial membranes by quantifying the uptake of the fluorescent probe 1-N-pheynylapthylamide (NPN), as the mode of action of AMPs often involves their insertion into bacterial membranes. After addition of 10µM of NPN to immediate inoculates of bacteria and CM (50 million CFU in 150µL), fluorescence readings (excitation 355nm, emission 444nm) were performed. Additionally, RT-PCR was performed on cell lysates of BMASC and BMF to identify the presence of selected β-defensins (DEFB1 and BNB3), cathelicidins (CAMP), and lactoferrin (LTF), all known and characterized AMPs in bovine serum or milk. Identified AMPs

were further studied using ELISAs to determine and quantify their presence in the CM of BMaSC or BMF. Three independent replicates were performed for each experiment. ANOVAs with Tukey's pairwise comparisons were used to evaluate statistical differences using JMP.

Results

A concentration-dependent bactericidal effect was found for serial dilutions of CM with the greatest reduction of 80% of bacterial growth relative to the control. No statistical differences were found between CM from BMaSC and BMF. No additional reduction in bacterial growth was found when using CM of pretreated cells versus the CM of untreated cells. Membrane permeability assays showed that bacteria treated with BMaSC CM and BMF CM had greater fluorescence than the negative acellular and penicillin-streptomycin controls (15493, 11539, 1716, and 1802 arbitrary units, respectively; $P<0.05$), indicating immediate and direct targeting of bacterial membranes rather than targeting of membrane synthesis. Using RT-PCR, mRNA of the likely causative AMPs DEFB1, BNB3, LTF and CAMP were detected in cell lysates of both BMaSC and BMF. Bands on the agarose gel were more intense for the β-defensins DEFB1 and BNB3 in BMaSC vs. BMF. When the AMP products in CM were quantified by ELISA, amounts of LTF were greater in CM from BMaSC than BMF (0.92ng/mL vs 0.56ng/mL, $P<0.01$) but much lower than levels described in bovine milk (50.5 µg/mL). The AMPs DEFB1 and CAMP were undetectable (<0.156 ng/mL, <2.5 ng/mL, respectively) in the CM of both BMaSC and BMF, even after concentrating CM.

Conclusion

Secreted factors, including the AMP lactoferrin, from both bovine mammary stem/precursor cells (BMaSC) and primary mammary fibroblasts (BMF) were shown to have antimicrobial properties against the mastitis pathogen *K. pneumonia*, most likely by immediate and direct targeting of bacterial membranes rather than membrane synthesis. Further experiments are focused on the identification of additional AMPs in CM that have been previously documented in milk and serum. The outcomes of this research might have meaningful impacts on the current treatment regiments for *K. pneumonia* mastitis.

References

Schukken Y., M. Chuff, P. Moroni, A. Gurjar, C. Santisteban, F. Welcome, and R. Zadoks. 2012. The "other" gram-negative bacteria in mastitis: Klebsiella, serratia, and more. Vet. Clin. North Am. Food Anim. Pract. 28: 239-56.

Sharma, N., and D.K. Jeong. 2013. Stem cell research: a novel boulevard toward improved bovine mastitis management. Int. J. Biol. Sci. 9(8):818-29.