Udder Health and Milk Quality Management: Challenges and Opportunities under Flemish Conditions

Pieter Passchyn

Torhout, 2016

“It is not the strongest of the species that survives,

nor the most intelligent that survives.

It is the one that is most adaptable to change”

Charles Darwin
(1809 – 1882)

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List of Abbreviations

AMS	automated milking system
BC	bacterial count
BMSCC	bulk milk somatic cell count
CC	coliform count
CFU	colony forming units
CM	clinical mastitis
CNS	coagulase-negative staphylococci
DHI	dairy herd improvement
DIM	days in milk
EEC	European economic community
EICM	estimated incidence of clinical mastitis
hMQP	hypothetical milk quality premium
HPLC	high performance liquid chromatography
IBC	individual bacterial count
IM	intramuscular
IMI	intramammary infection
IQR	inter quartile range
IRCM	incidence rate of clinical mastitis
LnSCC	natural log-transformed somatic cell count
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MQP	milk quality premium
MY	milk yield
PMTD	postmilking teat disinfection
SCC	somatic cell count
TBC	total bacterial count
VHHM	veterinary herd health management

Chapter 1

General Introduction

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Background

Milk and derived dairy products are essential sources of food for the majority of the world population. The per capita consumption of milk and dairy products is higher in developed countries, but the gap with many developing countries is narrowing. The growing demand offers a good opportunity for producers in high-potential, peri urban areas to enhance their livelihoods through increased production (FAO, 2015). In 2014, the EU member states were delivering 40% of the global milk production. Also in Belgium the total milk production has increased with almost 20% since 2006 (Annual Report BCZ, 2015). Within Belgium, Flemish dairy farms are responsible for this increase (Annual Report BCZ, 2015). On the other hand, there are substantial differences between the Flemish provinces, with the largest herds being located in the provinces of Antwerp and Limburg, and the smallest herds in Vlaams-Brabant and West-Vlaanderen. However, the largest milk deliveries originate from the province West-Vlaanderen (Annual Report BCZ, 2015). In order to achieve this growth and to keep dairy farming profitable at the same time, the average milk yield (MY) per cow as well as the average herd size, has increased substantially in recent decades (Lucy, 2001). The higher MY has resulted from genetic selection as well as improved cow nutrition and management (Barkema et al., 2015). Also, while in the past heifer rearing was too often neglected on dairy farms, it has become a priority in modern intensive dairy systems. Huijps and Hogeveen (2007) illustrated that a heifer producing 8,000 kg of milk during the first lactation will have returned little more than the investment cost, provided it remains healthy during its first lactation, under Dutch (milk quota) circumstances. One of the diseases potentially threatening these challenges is mastitis. Besides well-studied mastitis pathogens such as *Staphylococcus aureus*, *Escherichia coli* and the *Streptococcus uberis*, emerging pathogens causing mastitis are diagnosed more often than before, e.g. *Mycoplasma bovis* (Fox et al., 2005). Still, the prevalence on Flemish dairy herds is yet unknown. Also, together with the intensification on dairy farms in the last decades, the quality and safety of dairy products have become an increasing issue of concern for both consumers and producers (Flores-Miyamoto et al., 2014). This phenomenon is at least partly attributable to incidents such as the dioxin contamination of animal feed in Belgium (Boor, 2001; Noordhuizen and Metz, 2005) and Germany (Velthuis and Van Asseldonk, 2011), melamine-contaminated powdered infant formula in China (Haenlein, 2002; WHO, 2008), and outbreaks of *Escherichia coli* O104:H4 infection in 16 countries in Europe and North America (EFSA, 2010). Also, antimicrobial overuse and/or misuse in food animal production has the potential to increase antimicrobial resistance which might threat human health. The latter stresses the importance of the absence of residues in milk and even more the request for a more targeted use of antimicrobials in the dairy industry,

all the more so since approximately 80% of the antimicrobial residue violations in milk can be traced back to mastitis treatments (Leslie et al., 1997; Ruegg and Tabone, 2000).

Udder health

Clinical and subclinical mastitis

Mastitis is defined as an inflammatory reaction of the udder tissue, generally in response to a bacterial intramammary infection (IMI) (Bradley, 2002). It is still one of the most costly diseases for the dairy industry as it affects a high proportion of dairy cows throughout the world. A recent study estimated the costs of mastitis at 140 euro per cow per year (Halasa et. al., 2009).

The disease is a 2-faceted health problem including subclinical and clinical mastitis (CM) (Barnouin et al., 2005). Clinical mastitis is readily apparent and easy to detect by the milker - less so by an automated milking system (AMS) - as it results in alterations in milk appearance (clots, flakes, watery secretion,...) and a decreased milk production, often accompanied with elevated body temperature, and swelling, redness, pain or heat of the infected udder quarter(s). Subclinical mastitis, which is the most prevalent form of mastitis, reduces the milk production and milk quality without visible signs. The most common method to detect cases of subclinical mastitis is by measuring the milk somatic cell count (SCC). An elevated SCC is a sign of inflammation and a useful indicator for the presence of IMI (Schukken et al., 2003). The bulk milk somatic cell count (BMSCC) is, besides being a proxy for the prevalence of cows suffering from subclinical mastitis in a herd, also a key milk quality parameter in national and international regulatory systems.

Mastitis causing pathogens

Various pathogens, typically bacteria, are capable of invading the mammary gland, to multiply, and to induce an inflammatory response. Over 200 different organisms have been recorded in scientific literature as potential causes of bovine mastitis (Blowey and Edmondson, 2010). Mastitis-causing pathogens can be classified as either environmental or contagious in nature, reflecting their epidemiological behaviour (Table 1). So-called environmental pathogens are particularly adapted to survive in the bovine environment and can be considered opportunistic invaders of the mammary gland. The most frequently isolated environmental pathogens are streptococci other than *Streptococcus agalactiae*, commonly referred to as environmental streptococci, and Gram-negative bacteria such as *E. coli* and *Klebsiella* spp. (Bramley and Dodd, 1984; Smith et al., 1985). So-called contagious mastitis pathogens are adapted to survive within the mammary gland and are spread from cow to cow primarily during the milking

process. Examples are: *S. aureus*, *Strep. agalactiae*, *Mycoplasma* species, and *Corynebacterium bovis* (Smith, 1983; Bramley and Dodd, 1984). However, this classification is not as clear-cut as accepted before as strains within species can act differently (Zadoks and Schukken, 2006). Classifying mastitis causing pathogens as purely environmental or contagious is, however, a simplified representation of the reality. Evidence has been provided for both environmental *S. aureus* as well as contagious *Strep. uberis* mastitis-causing strains (Zadoks et al., 2001, 2002a). Therefore, the epidemiology of mastitis-causing pathogens is more and more represented by a sliding scale, where the balance of contagious and environmental transmission shifts gradually, instead of a species-based dichotomy (Zadoks, 2002b). The sources and transmission routes of the coagulase-negative staphylococci (CNS) are currently still largely unknown. In a recent study, they appeared to be more environmental as group than contagious (Sampimon et al., 2009), yet differences between species have been reveald (De Visscher et al., 2014; Vanderhaeghen et al., 2015).

Table 1. Overview of the most prevalent mastitis pathogens, classified as environmental or contagious.

Contagious pathogens	**Environmental pathogens**
Staphylococcus aureus	*Streptococcoccus uberis*
Streptococcus agalactiae	Coliforms (*E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp.)
	Bacillus cereus, Bacillus licheniformis
Streptococcus dysgalactiae	*Pasteurella* spp.
	Pseudomonas aeruginosa
Corynebacterium bovis	*Streptococcus faecalis*
Mycoplasma spp.	Fungi, Yeasts

Recently, mastitis caused by *Mycoplasma* spp. have been receiving more attention. The premise that Mycoplasma mastitis has an emerging presence is reflected in reports from Canada (Francoz et al., 2012), Greece (Fillioussis et al., 2007), Iran (Ghazaei, 2006), and Mexico (Miranda-Morales et al., 2008) and the multifold increase in herds with apparent Mycoplasma mastitis over time in the Pacific Northwest of the USA (Fox et al, 2003). *Mycoplasma bovis* is clearly the most prevalent *Mycoplasma* spp. that causes mastitis and it is estimated that more than 90% of the Mycoplasma mastitis cases are due to *Mycoplasma bovis* (Boonyayatra et al., 2012). Other *Mycoplasma* spp. that cause mastitis include: *Mycoplasma californicum*, *Mycoplasma bovigenitalium*, *Mycoplasma arginini*, *Mycoplasma bovirhinis*, *Mycoplasma canadense*, and *Mycoplasma alkalescens* (Gonzalez and Wilson, 2003). One of the explanations for the emerging nature of Mycoplasma mastitis might be found

in the increasing herd size. There is a significant correlation between herd size and the risk of Mycoplasma mastitis (Thomas et al., 1981; Fox et al., 2003). Presumably, this is because larger herds are more likely to import new cattle. It is assumed that the increased risk is a result of the inclusion of cattle that are infected with novel strains of *Mycoplasma* spp., where such infection might be symptomatically or asymptomatically expressed (Fox, 2012). However, the results reported by Wilson et al. (2007) indicate that an outbreak of Mycoplasma-associated disease could also occur spontaneously in a closed herd. Risk factors other than novel introduction of the agent might thus exist as well. Until now, no information is available on the prevalence of Mycoplasma mastitis on Flemish dairy herds.

Prevention and control

Mastitis prevention and control programs were developed in the sixties of the last century and have ever since been adopted with considerable success in reducing the prevalence and incidence of subclinical and clinical mastitis in every dairy herd where it has been applied (Hillerton et al., 1995; Bradley, 2002). The first standard mastitis control program comprised 5 points: appropriate treatment of clinical mastitis, culling of chronically infected cows, post-milking teat disinfection, correct maintenance and use of the milking equipment, and application of blanket dry cow therapy (Neave et al., 1969). Those measures mainly focused on how to reduce the transmission of contagious mastitis pathogens, and thus were less effective against environmental pathogens. Studies have shown that as the prevalence of contagious mastitis pathogens is reduced, the proportion of mastitis cases caused by environmental pathogens increases (Oliver et al., 1997; Jayarao et al., 1999). It was therefore realised that with these shifting distributions of pathogens, new approaches were needed. This eventually resulted in the extension of the 5-point plan into the 10-point prevention and control program of the National Mastitis Council (2014). The first points of the program still focus on the prevention and control of contagious mastitis pathogens. The added points stress the importance of a comfortable, hygienic and well-ventilated housing to prevent and control mastitis caused by environmental pathogens, besides some other aspects related to goal setting and regular monitoring. However, the shift in the distribution of mastitis pathogens is still continuing and has in many countries resulted in a substantial increase of CNS IMI over the last 10 years (Myllys et al. 1998; Makovec and Ruegg, 2003; Pitkäla et al., 2004), also in Flanders (Piepers et al., 2007).

Interestingly, most measures included in the 10-point standard mastitis prevention and control program are derived from studies that focused on subclinical mastitis and not on CM. In that respect, it is surprising to see only a few peer-reviewed publications report on risk factors for CM (Peeler et al., 2000; Barnouin et al. 2005; Verbeke et al., 2014). Some studies suggested that low BMSCC was associated with a high incidence rate of CM (IRCM) (Elbers

et al., 1998; Beaudeau et al., 2002; Green et al., 2004) whereas other work did not reveal any interrelationship (Barkema et al., 1998; Beaudeau et al., 1998). In general, there is a lack of information on success factors for herds combining a low incidence rate of clinical mastitis (IRCM) with a low BMSCC, also in our part of the world.

The increase in average herd size has required an increase in the number of replacement heifers, prioritizing optimal dairy heifer rearing in modern intensive dairy systems. A heifer has returned little more than the investment cost after completion of the first lactation, provided it has remained healthy. One of the diseases threatening its health is mastitis. It is currently well-known that a high proportion of heifers freshens with IMI in one or more quarters (De Vliegher et al., 2012). Mastitis in dairy heifers is recognised as a distinct problem from that in multiparous cows because of its different pattern of disease and unique factors that determine its occurrence. After all, primigravid animals have never been milked upon calving whereas the milking process is, as mentioned above, generally considered one of the principal risk factors of mastitis. Consequently, heifers are less exposed to contagious pathogens and their teats have not been challenged by the milking vacuum, undermining its role as first barrier against environmental pathogens. All in all, heifer mastitis is a costly disease. Typically, the cost of mastitis is ascribed to lost milk production, premature culling, additional labor, management changes, and veterinary needs, additional use of drugs and increased risk of residues, and production of nonsaleable milk (Huijps et al., 2009). Early lactation mastitis in heifers also results in lost future milk yield (Coffey et al., 1986; Rupp and Boichard, 2000; De Vliegher et al., 2005a). Additionally, heifer mastitis increases the risk of premature culling (De Vliegher et al., 2005b) and puts heifers at greater risk for CM (Coffey et al., 1986; Rupp and Boichard, 2000). More cases of CM in its turn is associated with an increased drug use, more discarded milk, and an increased risk of residues appearing in the milk supply. Several pathogens are involved in heifer mastitis, with coagulase-negative staphylococci (CNS) being the most prevalent (Fox, 2009; De Vliegher et al., 2012). Recent work suggests that CNS IMI in fresh heifers have a less pronounced effect on the heifers' future performances compared with IMI caused by major pathogens, making the need for prevention of IMI with CNS, at least in early lactation heifers, not a priority, or even unwanted, as heifers with CNS IMI at calving produce more and have a lower IRCM during their first lactation compared with noninfected heifers (Piepers et al., 2010, 2013; Pearson et al., 2013). The high prevalence of IMI in fresh dairy heifers and the potentially compromising effects for future productivity have urged researchers to seek for risk factors specifically associated with mastitis in dairy heifers. Based on the findings of all heifer mastitis risk factor studies conducted in the last decades, a 10-point program specifically focusing on the prevention and control of heifer mastitis was recently proposed (De Vliegher et al., 2012), but did not distinguish between (subgroups of) mastitis

pathogens (Table 2). Still, studying pathogen group-specific risk factors for IMI in early lactating heifers is even more interesting as it allows for the development of pathogen group-specific prevention and control programs.

Table 2. Ten-point program to prevent and control heifer mastitis (De Vliegher et al., 2012 and NMC, 2014).

1. Improve general udder health management at the farm level to decrease the pressure of infection with udder pathogens from older cows to heifers
2. Control for cross-suckling in calves and young stock
3. Implement an effective and efficient fly control system
4. Keep young and primigravid heifers in a clean and hygienic environment and separate from multiparous animals—provide as much attention to this group of animals related to hygiene and cleanliness as is spent on lactating animals
5. Avoid any nutritional deficiency—monitor vitamin E and selenium levels when any doubt exists, especially in relation to CM; zinc, copper, and vitamin A play a role as well and could be checked
6. Minimize the risk of negative energy balance before and after calving through appropriate transition feeding systems
7. Reduce the incidence of udder edema through optimized peripartum management
8. Minimize stress around calving (e.g., by not moving heifers to the calving pen when already in labor) and minimize incidence of dystocia and peripartum disease
9. Consider use of internal teat sealants prepartum where a high risk of environmental mastitis exists in the peripartum period
10. Use prepartum antibiotic treatment in heifers under certain conditions only:
a.under the supervision of the herd veterinarian, within the context of a valid veterinary/client/patient relationship
b.after quantification of the problem and identification of major pathogens (not CNS) as the cause through culturing
c.choice of the antibiotics should be based on antimicrobial susceptibility testing
d.testing for residues before every milk delivery
e.upgrading of management at the same time—discontinue treatment as soon as new management strategies become effective

Milk quality and milk safety

Microbiological contamination of milk is an important issue because pathogens can affect food safety, and spoilage microorganisms can limit shelf life and affect quality or yield of dairy products. Bacterial milk quality can be determined using several parameters including bacterial count (BC), preliminary incubation counts, laboratory pasteurization counts, and coliform counts (CC; Murphy, 1997). Among these, BC is the most commonly used one in regulatory programs (Murphy and Boor, 2000). Besides penalties for not meeting the legal milk quality standards, the majority of milk processors in Flanders (Belgium) pays incentives to farmers that meet higher quality requirements. The combination of a system combining both penalties and premiums has shown to provide a strong incentive for improvement of milk quality (Nightingale et al., 2008) and is also a strong motivator to implement management changes on dairy farms. In Flanders (Belgium), the official mandatory milk quality regulations follow European legislation and require a geometric mean BMSCC over the last three months (based on 4 recordings per month) < 400 x 10^3 cells/mL, a geometric mean BC, expressed as individual bacterial count (IBC) over the last two months (based on two recordings per month) < 100 x 10^3 IBC/mL milk, a geometric mean of the freezing point over the last two months (based on all deliveries) > -510 m°C, no visible impurities (filtration test done once a month) and absence of antibiotic residues in any milk delivery. In 2014, 2.75% of the herds received one or more penalties because of not meeting the legal standards (Annual Report MCC Vlaanderen, 2015). In contrast, testing of CC is nonobligatory for milk quality in Flanders, yet implemented as part of the aforementioned incentive program. However, there is a lack of information on factors related to (not) achieving milk quality premiums, also in Flanders. With a low milk price and marginal profits, it is important to provide farmers with tools to produce high quality milk in the most efficient way. Until now, most studies have identified factors holding information on milking and equipment hygiene, sanitizing procedures (Elmoslemany et al., 2009; Pantoja et al., 2011), and milk storage conditions (Murphy and Boor, 2000), explaining variability in TBC and CC. Apart from 2 studies (van Schaik et al., 2005 and Elmoslemany et al., 2010), factors related to either herd health management, transition and feeding management, or housing, which are known to affect udder health, have not been studied as potential factors related to milk quality. Also, little information is available on which management practices predispose a farm to the loss of premiums due to inferior milk quality.

A shift from curative towards preventive veterinary medicine

With regard to the herd size and management of dairy farms over the past few decades, some trends have become visible. Dairy farms have been coping with increased (feed) costs and had to improve productivity for that reason or changed their business model towards e.g. organic farming. Apart from having more cows, cows have also been genetically selected to produce more milk (Noordhuizen and Wentink, 2001). This intensification led to more cows per producer and more production-related problems such as subfertility and subclinical disease (Shanks et al., 1978). The focus of dairy management has therefore changed from curative to preventive (Cannas da Silva et al., 2006; LeBlanc et al., 2006). Yet, even though modern dairy producers are nowadays more aware of the costs of animal diseases than some decades ago and are therefore more eager to prevent them, they often experience difficulties with their detection and with finding a solution for them (Cannas da Silva et al., 2006). Both scientific evidence and personal experiences from the field teach that farmers therefore often keep returning their old habits and keep reaching for antimicrobials in case of (udder/infectious) health problems rather than changing their herd management in a way that disease can be prevented in the future (McDougall et al., 2009). Still, in order to safeguard the effectiveness of existing and novel antimicrobials, there is an increasing global pressure to develop strategies in both human and veterinary medicine to slow down the emerging resistance in bacteria by selection pressure (Morley et al., 2005). This implies a shift to a more preventive rather than curative veterinary medicine and a more responsible use of drugs when antimicrobials are needed.

Prevention and treatment of mastitis accounts for 60% of the antimicrobial use on a dairy farm of which approximately 50% is applied for (blanket) dry cow therapy (Stevens et al., 2016). Treatment of all cows with long-acting antimicrobials at dry-off (so-called blanket dry cow therapy) has been applied since the 1960 (Neave et al., 1969) and is still a cornerstone of the 10-point mastitis prevention and control program (NMC, 2009). In the early 1990ies, prepartum antimicrobial treatment of end-term heifers was also suggested in the control of heifer mastitis. In general, prepartum treatment of heifers with lactating or dry cow products results in higher bacteriological cure rates of infected quarters (Nickerson et al., 2009). Studies investigating the effects of systemic treatment are, however, rare (Kreiger et al., 2007; Parker et al., 2008), although systemic over local antibiotic treatment has the advantages of a decreased risk of teat contamination (i.e. iatrogenic infection) during the application, is more convenient and safer to administer, and allows to treat four quarters at a time with a single administration. Logically, a widespread distribution of the systemically administered antimicrobial drug in the dry udder is a prerequisite for being successfull. Positive long-term effects of prepartum

antibiotic treatment such as lower SCC and higher MY over the first lactation were seen in some studies (e.g. Sampimon et al., 2009) but not in others (Borm et al., 2006). The latter indicates that some yet not identified herd-specific factors (e.g. prevalence of major pathogens, milk production, …) might play a role. Also, the added value of treatment over the implementation of other prevention and control measures, in particular on farms that do not specifically suffer from a true heifer mastitis problem, is yet not known. Finding an answer on the latter question has even become more relevant over the last few years as the use of antimicrobials in farming animals is increasingly subjected to criticism (Kaesbohrer et al., 2012).

Monitoring and managing herd health, such as udder health, has become an important and challenging issue on dairy farms. To cope with these challenges, veterinary herd health management programs (VHHM) were set up. They are defined as a combination of (advice on) animal health, milk production, and disease prevention, placed in a framework of farm economics, welfare, food safety, and environment (Brand et al., 1996). Given their knowledge on epidemiology, farm management, and physiology and pathology of dairy cows, veterinary practitioners have always been an important partner for dairy farmers regarding animal health and herd profitability. This started in the 1960ies with mastitis control (Bramley and Dodd, 1984), followed by herd fertility schemes (Bramley and Dodd, 1984; Esslemont et al., 1985; Esslemont et al., 2001), disease prevention (de Kruif and Opsomer, 2004) and, finally, quality control programs (Esslemont et al., 1985; Noordhuizen and Wentink, 2001; de Kruif and Opsomer, 2004). Veterinary herd health management programs ideally follow a fixed structure of goal setting, advice, action, and evaluation (Brand et al., 1996) as shown in Figure 1. Defining short- and long-term goals and getting a better understanding of the motivation and mindset of each individual farmer is a first step in such a VHHM program. Some farmers perceive the extra stress and labour as the most annoying aspects of mastitis while other farmers are more worried by the lost revenues. Also, the level at which mastitis is perceived as a problem and thus the goals will differ between farmers (Huijps et al., 2008). Of course, in the light of a more prudent use of antimicrobials, it would be good if dairy farmers became aware of potential problems with infectious disease and are open for possible improvements before serious problems occur (Lam et al. 2011). A more pro-active rather than reactive role on the part of the veterinarian in supporting the dairy farmer could of course be very helpful. Also, while defining the farmer-specific goals, the interests of both the milk buyers and the society should not be forgotten. In a second step, data should be collected, recorded and analysed. In a third step, herd-specific advices should be formulated and a farmer-tailored (udder) health management plan should be implemented. And last but not least, results should be regularly evaluated and the formulated management plan should be adapted accordingly.

Still, to be succesfull, more insights are needed in the potential challenges and bottlenecks that one might encounter in setting-up and executing such a VHHM program, also when focussing on udder health and milk quality, under the current Flemish conditions, but which might, in turn, offer opportunities for both the vets and farmers (Fig. 1).

Conclusions

The dairy industry in the developed world has undergone profound changes over the recent decades (Barkema et al., 2014). Also in Flanders the average herd size has continuously increased with labour becoming more and more a limiting factor on the family-owned farms. Since the abolition of the quota system in Europe, the price producers receive for there milk is market-driven and highly volatile at times. At the same time consumers and citizens have long-standing interests in the safety and quality of milk products (Drake, 2007) and in the welfare of the cows that produce milk and its associated products (von Keiserlingk et al., 2009). Since livestock can be reservoirs of resistance genes (Fey et al., 2000), antimicrobial resistance in bacteria has become an important public hazard (Prescott, 2014).

Flemish dairy farmers and their veterinarians must learn how to deal with these challenges in the near future, if they want to retain their position in the global dairy industry.

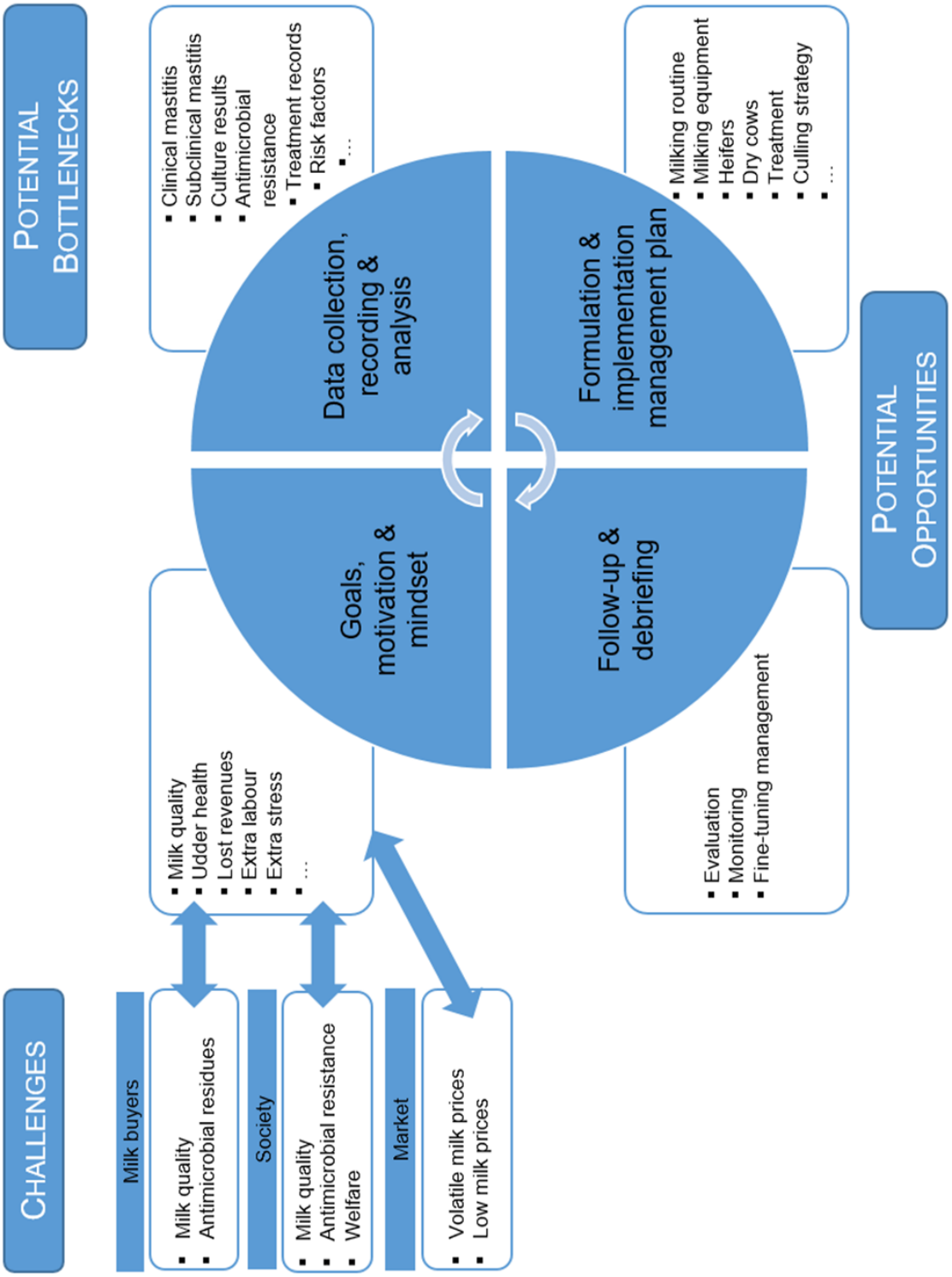

Figure 1. Veterinary herd health management programs follows a fixed structure (adapted from Brand et al, 1996).

References

Barkema, H. W., Y. H. Schukken, T. J. G. M. 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. 81:411-419.

Barkema, H. W., M. A. G. von Keyserlingk, J. P. Kastelic, T. J. G. M. Lam, C. Luby, J.-P. Roy, S. J. LeBlanc, G. P. Keefe, and D. F. Kelton. 2015. *Invited review*: Changes in the dairy industry affecting dairy cattle health and welfare. J. Dairy Sci. 98:7426–7445.

Barnouin, J., S. Bord, S. Bazin, and M. Chassagne. 2005. Dairy Management Practices Associated with Incidence Rate of Clinical Mastitis in Low Somatic Cell Score Herds in France. J. Dairy Sci. 88:3700-3709.

Beaudeau, F., C. Fourichon, H. Seegers, and N. Bareille. 2002. Risk of clinical mastitis in dairy herds with a high proportion of low individual milk somatic cell-count. Prev. Vet. Med. 53:43-54.

Belgian Dairy Confederation. 2015. Annual report. Belgian Dairy Confederation, Leuven, Belgium.

Blowey, R., and P. Edmondson. 2010. Mastitis Control in Dairy Herds. 2nd Edition. Page 36.

Boonyayatra, S., L.K. Fox, T.E. Besser, A. Sawant, J.M. Gay and Z. Ravivi. 2012. A PCR assay and PCR-Restriction fragment length polymorphism combination identifying the 3 primary Mycoplasma species causing mastitis. J. Dairy Sci. 95:196-205.

Boor, K. J. 2001. Fluid dairy product quality and safety: Looking to the future. J. Dairy Sci. 84:1-11.

Borm, A. A., L. K. Fox, K. E. Leslie, J. S. Hogan, S. M. Andrew, K. M. Moyes, S. P. Oliver, Y. H. Schukken, D. D. Hancock, C. T. Gaskins, W. E. Owens, and C. Norman. 2006. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. J. Dairy Sci. 89:2090-2098.

Bradley, A. J. 2002. Bovine mastitis: an evolving disease. Vet. Journal 164:116-128.

Bramley, A.J. and Dodd, F.H. 1984. Reviews of the progress of dairy science: Mastitis control - progress and prospects. J. Dairy Res. 51: 481–512.

Brand, A., J. P. T. M. Noordhuizen, and Y. H. Schukken, ed. 1996. Herd health and production management in dairy practice. Wageningen Pers, Wageningen, the Netherlands.

Cannas da Silva, J., J. P. T. M. Noordhuizen, M. Vagneur, R. Bexiga, C. C. Gelfert, and W. Baumgartner. 2006. Veterinary dairy herd health management in Europe contstraints and perspectives. Vet. Q. 28:23-32.

Coffey E. M., Vinson W. E., and R.E. Pearson. 1986. Somatic cell counts and infection rates for cows of varying somatic cell count in initial test of first lactation. J. Dairy Sci. 69:552-555.

de Kruif, A., and G. Opsomer. 2004. Integrated dairy herd health management as the basis for prevention. Vlaam. Diergeneeskd. Tijdschr. 73:44-52.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2005a. Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. J. Dairy Sci. 88:938-947.

De Vliegher, S., H. W. Barkema, G. Opsomer, A. de Kruif, and L. Duchateau. 2005b. Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. J. Dairy Sci. 88:560-568.

De Vliegher, S., L.K. Fox, S. Piepers, S. McDougall, and H.W. Barkema. 2012. Invited review: mastitis in dairy heifers. J. Dairy Sci. 95 :1025–1040.

Drake, M. A. 2007. *Invited review*: Sensory analysis of dairy foods. J. Dairy Sci. 90:4925–4937.

De Visscher, A., K. Supré, F. Haesebrouck, R. N. Zadoks, V. Piessens, E. Van Coillie, S. Piepers S, and S.De Vliegher. 2014. Further evidence for the existence of environmental and host-associated species of coagulase-negative staphylococci in dairy cattle. Vet Microbiol. 172(3-4):466-74.

EFSA (European Food Safety Authority). 2010. The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA J. 8: 1496–1906

Elbers, A. R. W., J. D. Miltenburg, D. De Lange, A. P. P. Crauwels, H. W. Barkema, and Y. H. Schukken. 1998. Risk factors for clinical mastitis in a random sample of dairy herds from the southern part of The Netherlands. J. Dairy Sci. 81:420-426.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, and B. M. Jayarao. 2009. Risk factors for bacteriological quality of bulk tank milk in Prince Edwards Island dairy herds. Part 1: Overall risk factors. J. Dairy Sci. 92:2634-2643.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, J. J. Wichtel, H. Stryhn, and R. T. Dingwell. 2010. The association between bulk milk analysis for raw milk quality and on-farm management practices. Prev. Vet. Med. 95:32-40.

Esslemont, R.J., Bailie, J.H., and Cooper, M.J. 1985. Fertility Management in Dairy Cattle. Collins Professional and Technical Books, London, UK

Esslemont, R.J., Kossaibati, M.A., and Allcock, J. 2001. Economics of fertility in dairy cows. Br. Soc. Anim. Sci. Occ. Publ. 26: 19–29.

FAO. Food and agriculture Organization of the United Nations. 2015. http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/en/#.VcS6oCbtlBc

Fey, P. D., T. J., Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs. 2000. Ceftriaxone-resistant Salmonella infection acquired by a child from cattle. N. Engl. J. Med. 342:1242–1249.

Filioussis, G, G Christodoulopoulos, A Thatcher, V. Petridou , and E. Bourtzi-Chatzopoulou.. 2007. Isolation of Mycoplasma bovis from bovine clinical mastitis cases in Northern Greece. Vet. J.;173:215-218.

Flores-Miyamoto, A., M.W. Reij, and A.G.J Velthuis. 2014. Do farm audits improve milk quality? J. Dairy Sci. 97:1-12.

Fox, LK, Hancock, DD, Mickelson, A, and A. Britten. 2003. Bulk tank milk analysis: Factors associated with appearance of Mycoplasma sp. in milk. J. Vet. Med B 50:235-240.

Fox, L. K., J. H. Kirk, and A. Britten. 2005. Mycoplasma mastitis: a review of transmission and control. J. Vet. Med. 52:153-160.

Fox, L. K. 2009. Prevalence, incidence and risk factors of heifer mastitis. Vet. Microbiol. 134:82-88.

Fox, L.K. 2012. Mycoplasma mastitis: Causes, Transmission and Control. Mastitis, Veterinary Clinics of North America: Food Animal Practice. Pp. 225-237. P. Ruegg (ed). Vol. 28, 2, W.B. Saunders Co., Philadelphia.

Francoz, D., L. Bergeron, M. Nadeau, and G. Beauchamp. 2012. Prevalence of contagious mastitis pathogns in bulk tank milk in Quebec. Can. Vet. J. 53:1071-1078.

Ghazaei, C. Mycoplasmal mastitis in dairy cows in the Moghan region of Ardabil State, Iran. 2006. J. S. Afr. Vet. Assoc. 77:222-223.

Gonzalez, R.N. and D.J. Wilson. 2003.Mycoplasma mastitis in dairy herds. Vet Clin North Am Food Anim Pract;19:199-221.

Green, M. J., P. R. Burton, L. E. Green, Y. H. Schukken, A. J. Bradley, E. J Peeler, and G. F. Medley. 2004. The use of Markov Chain Monte Carlo for analysis of correlated binary data: Patterns of Somatic Cells in Milk and the Risk of Clinical Mastitis in Dairy Cows. Prev. Vet. Med. 64:157-174.

Haenlein, G.F.W. 2002. Relationship of somatic cell counts in goat milk to mastitis and productivity. Small Rumin. Res. 45: 163–178

Halasa, T., Nielen, M., Huirne, R.B.M., and Hogeveen, H. 2009. Stochastic bio-economic model of bovine intramammary infection. Livest. Sci. 124: 295–305.

Hillerton, J. E., A. J. Bramley, R. T. Staker, and C. H. McKinnon. 1995. Patterns of intramammary infection and clinical mastitis over a 5 year period in a closely monitored herd applying mastitis control measures. J. Dairy Res. 62:39-50.

Huijps, K., and H. Hogeveen. 2007. Stochastic modelling to determine the economic effects of blanket, selective and no dry cow therapy. J. Dairy Sci. 90:1225-1234.

Huijps, K., S. De Vliegher, T. Lam, and H. Hogeveen. 2009. Cost estimation of heifer mastitis in early lactation by stochastic modelling. Vet. Microbiol. 134:121-127.

Jansen, J., C. D. M. Steuten, R. J. Renes, N. Aarts, and T. J. G. M. Lam. 2010. Debunking the myth of the hard-to-reach farmer: effective communication on udder health. J. Dairy Sci. 93:1296-1306.

Jayarao, B. M., B. E. Gillespie, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 1999. Epidemiology of Streptococcus uberis intramammary infections in a dairy herd. J. Vet. Med. B. 46:433-442.

Kaesbohrer, A., A. Schroeter, B. A. Tenhagen, K. Alt, B. Guerra, and B. Appel. 2012. Emerging antimicrobial resistance in commensal Escherichia coli with public health relevance. Zoonoses Public Hlth 59: 158-165 Suppl. 2.

Kreiger, M., G. Friton, J. Hofer, K. Fuchs, and P. Winter. 2007. Effects of periparturient systemic treatment with penethamate hydriodide on udder health and milk yield of dairy heifers. J. Dairy Res. 74:392-398.

Lam, T. J. G. M., J. Jansen, B. H. P. van den Borne, R. J. Renes, and H. Hogeveen. 2011. What veterinarians need to know about communication to optimize their role as advisors on udder health in dairy herds. New Zealand Vet. J., 59:8-15.

LeBlanc, S.J., Lissemore, K.D., Kelton, D.F., Duffield, T.F., and Leslie, K.E. 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89: 1267–1279.

Leslie, K. E., Y. H. Schukken, J. Sargeant, and M. Mitchell. 1997. Inhibitor Violations during 10 years in Ontario. Association with Somatic Cell Count Reduction Program. Report to the Ontario Buro of Veterinary Drugs.

Lucy, M. C. 2001. Reproduction loss in high-producing dairy cattle: Where will it end? J. Dairy Sci. 84: 1277-1293.

Makovec, J. A., and P. L. Ruegg. 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J. Dairy Sci. 86:3466-3472.

McDougall, S., K. I. Parker, C. Heuer, and C. W. R. Compton. 2009. A review of prevention and control of heifer mastitis via non-antibiotic strategies. Vet. Microbiol. 134:177-185.

Milk Control Centre Flanders. 2015. Annual Report. Milk Control Center Flanders, Lier, Belgium.

Miranda-Morales, RE, V Rojas-Trejo, R Segura-Candelas, E.M. Carillo-Casas, M.G. Sanchez-Gonazlez, R.S. Castor, and F.J. Trigo-Tavera. 2008. Prevalence of pathogens associated with bovine mastitis in bulk tank milk in Mexico. Ann. N.Y. Acad. Sci.;1149:300-302

Morley, P. S., M. D. Apley, T. E. Besser, D. P. Burney, P. J. Fedorka-Cray, M. G. Papich, J. L. Traub-Dargatz, and J. S. Weese. 2005. Antimicrobial drug use in veterinary medicine. J. Vet. Intern. Med. 19(4):617-629.

Murphy, S.C. 1997. Raw milk bacteria tests: Standard plate count, preliminary incubation count, lab, pasteurization count and coliform count. What do they mean for your farm?

Pages 34-41 in Natl. Mastitis Council. Reg. Mtg. Proc., Syracuse, New York. Natl. Mastitis Council, Inc., Madison, WI.

Murphy, S. C., and K. J. Boor. 2000. Trouble-shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ. Sanit. 20:606-611.

Myllys, V., K. Asplund, E. Brofeldt, V. Hivelä-Koski, T. Honkanen-Buzalski, J. Junttila, L. Kulkas, O. Myllykangas, M. Niskanen, H. Saloniemi, M. Sandholm, and T. Saranpää. 1998. Bovine mastitis in Finland in 1988 and 1995 – Changes in prevalence and antimicrobial resistance. Acta Scand. Vet. 39:119-126.

Nickerson SC 2009 Control of heifer mastitis: antimicrobial treatment – An overview. Veterinary Microbiology 134 128-135

Nightingale, C., K. Dhuyvetter, R. Mitchell, and Y.H. Schukken. 2008. Influence of variable milk quality premiums on observed milk quality. J. Dairy Sci. 91:1236-1244.

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.

National Mastitis Council. (2009). Recommended mastitis control program, Madison WI, USA: National Mastitis Council Inc. http://www.nmconline.org/docs/NMCchecklistInt.pdf.

NMC 2014. http://nmconline.org/articles/heifer10pt.htm

Noordhuizen, J. P. T. M., and G. H. Wentink. 2001. Developments in veterinary herd health programmes on dairy farms: a review. Vet. Q. 23:162-169.

Noordhuizen, J.P.T.M. and Metz, J.H.M. 2005. Quality control on dairy farms with emphasis on public health, food safety, animal health and welfare. Livest. Prod. Sci. 94: 51–59.

Oliver, S. P., L. F. Calvinho, and R. A. Almeida. 1997. Characteristics of environmental Streptococcus species involved in mastitis. Pages 1–35 in Proc. Symp. Udder Health Mgmt. for Environ. Streptococcus, Guelph, Ontario, Canada.

Pantoja, J. C. F., D. J. Reinemann, and P. L. Ruegg. 2011. Factors associated with coliform count in unpasteurized bulk milk. J. Dairy Sci. 94: 2680-2691.

Parker, K. I., C. W. Compton, F. M. Anniss, C. Heuer, and S. McDougall. 2008. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic, or both, precalving. J. Dairy Sci. 91:169-181.

Pearson, L. J., J. H. Williamson, S. A. Turner, S. J. Lacy-Hulbert, and J. E. Hillerton. 2013. Peripartum infection with Streptococcus uberis but not coagulase-negative staphylococci reduced milk production in primiparous cows. J. Dairy Sci. 96:158-164.

Peeler, E. J., M. J. Green, J. L. Fitzpatrick, K. L. Morgan, and L. E. Green. 2000. Risk Factors associated with Clinical Mastitis in Low Somatic Cell Count British Dairy Herds. J. Dairy Sci. 83:2464-2472.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H. W. Barkema, and S. De Vliegher. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J Dairy Res. 74(4):478-83.

Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than non-infected heifers. J. Dairy Sci. 93: 2014-2024.

Piepers, S., Y. H. Schukken, P. Passchyn, S. De Vliegher. 2013. The impact of intramammary infection with coagulase-negative staphylococci in early lactating heifers on milk yield throughout first lactation revisited. J. Dairy Sci. 96:5095-5105.

Pitkälä, A., M. Haveri, S. Pyörälä, V. Myllys, and T. Honkanen-Buzalski. 2004. Bovine mastitis in Finland 2001 – Prevalence, distribution of bacteria, and antimicrobial resistance. J. Dairy Sci. 87:2433-2441.

Prescott, J. F. 2014. The resistance tsunami, antimicrobial stewardship, and the golden age of microbiology. Vet. Microbiol. 171:273–278.

Ruegg, P. L., and T. J. Tabone. 2000. The relationship between Antibiotic Residue Violations and Somatic Cell Counts in Wisconsin Dairy Herds. J. Dairy Sci. 83:2805-2809.

Rupp, R., and D. Boichard. 2000. Relationship of early first lactation somatic cell count with risk of subsequent first clinical mastitis. Livest. Prod. Sci. 62:169-180.

Sampimon, O. C., S. De Vliegher, H. W. Barkema, J. Sol, and T. J. G. M. Lam. 2009. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. J. Dairy Sci. 92:4395-4403.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky, and R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34:579-596.

Shanks, R.D., Freeman, A.E., Berger, P.J., and Kelley, D.H. 1978. Effect of selection for milk production and general health of the dairy cow. J. Dairy Sci. 61: 1765–1772.

Smith, K. L.. 1983. Mastitis control: a discussion. J. Dairy Sci. 66:1790-1794.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci 68:1531-1553.

Stevens, M., S. Piepers, K. Supré, J. Dewulf, and S. De Vliegher. 2016. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. J. Dairy Sci. 99:2118-2130.

Thomas, C.B., P.Willeberg, and D.E. Jasper. 1981. Case-control study of bovine mycoplasmal mastitis in California. Am . J . Vet . Res. 42:511-515.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, S. De Vliegher. 2015. Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants. Vet J. 203(1):44-51.

van Schaik G., L. E. Green, D. Guzman, E. Esparza, and N. Tadich. 2005. Risk factors for bulk milk somatic cell counts and total bacterial counts in smallholder dairy farms in the 10th region of Chile. Prev. Vet. Med. 67:1-17.

Velthuis, A. G. J., and M. A. P. M. van Asseldonk. 2011. Process audits versus product quality monitoring of bulk milk. J. Dairy Sci 94:235-249.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific Incidence Rate of Clinical Mastitis in Flemish Dairy Herds, Severity, and association with Herd Hygiene. J. Dairy Sci. 97:6926-6934.

von Keyserlingk, M. A., J. Rushen, A. M. de Passillé, and D. M. Weary. 2009. *Invited review*: The welfare of dairy cattle—Key concepts and the role of science. J. Dairy Sci. 92:4101–4111.

WHO. 2008. Melamine-Contaminated Powdered Infant Formula in China. World Health Organization, Geneva, Switzerland.

Wilson, D.J., R.T. Skirpstunas, J.D. Trujillo, K.B. Cavender, C.V. Bagley, and R. Harding, 2007. Unusual history and initial clinical signs of Mycoplasma bovis mastitis and arthritis in first-lactation cows in a closed commercial dairy herd. JAVMA 230:1519:1523.

Zadoks, R. N., and Y. H. Schukken. 2006. Use of molecular epidemiology in veterinary practice. Vet. Clin. Food Anim. 22:229-261.

Zadoks, R. N., H. G. Allore, H. W. Barkema, O. C. Sampimon, Y. T. Gröhn, and Y. H. Schukken. 2001. Analysis of an outbreak of Streptococcus uberis mastitis. J. Dairy Sci. 84:590-599.

Zadoks, R. N., H. G. Allore, T. J. Hagenaars, H. W. Barkema, Y. H. Schukken. A mathematical model of Staphylococcus aureus control in dairy herds. 2002a. Epidemiol. Infect. 129:397-416.

Zadoks, R.N. 2002b. Molecular and mathematical epidemiology of Staphylococcus aureus and Streptococcus uberis mastitis in dairy herds. Ph.D. Thesis, Utrecht University, Utrecht.

Sampimon, O. C., H. W. Barkema, I. M. G. A. Berends, J. Sol, and T. J. G. M. Lam. 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134:37-44.

Chapter 2

Aims and Outline of the Thesis

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The *general aim* of this thesis was to study (new) challenges related to udder health and milk quality (i.e. prudent use of antimicrobials, safeguarding milk quality and safety, excellent animal welfare, emerging pathogens, volatile milk prices, pressure to grow) that Flemish dairy farmers and their veterinarians are currently facing or will be facing in the (near) future. More specifically, this thesis aimed at giving better insights in if and how the udder health and milk quality on the Flemish dairy herds can be further improved in order to better respond to the public concerns related to antimicrobial usage, animal welfare, and milk quality and safety, and to at least partly counteract the volatile and often low milk prices. It also examines if mycoplasma mastitis is emerging on Flemish dairy farms as well, and which (additional) actions should be taken to further safeguard the udder health of their fresh dairy heifers.

In this regard, the *specific aims* of this thesis were:

- To develop a picture of the udder health and milk quality on Flemish dairy herds, using a number of parameters, i.e.:
    - The incidence of clinical mastitis and summary of the bulk milk somatic cell count **(Chapter 3.1)**,
    - The total bacterial count and coliform count in unpasteurized bulk milk **(Chapter 4.1)**,
    - Achieving milk quality premiums **(Chapter 4.2)**,
    - The between-herd prevalence of *Mycoplasma bovis* **(Chapter 3.2)**.

- To evaluate to what extent differences in management practices explain between-herd differences in those parameters, i.e.:
    - The incidence of clinical mastitis and the bulk milk somatic cell count **(Chapter 3.1)**,
    - The total bacterial and coliform count in unpasteurized bulk milk **(Chapter 4.1)**,
    - Achieving milk quality premiums **(Chapter 4.2)**.

- To evaluate the added value of systemically treating heifers prepartum with antimicrobials by:
    - Assessing the distribution of penicillin G in mammary tissue and secretions of heifers **(Chapter 5.1)**,
    - Assessing the short-term and long-term effects on udder health, milk yield, and culling **(Chapter 5.2)**,

- Identifying pathogen-group specific risk factors for intramammary infection in treated and untreated dairy heifers **(Chapter 5.3)**.

The results of this thesis give insights in which bottlenecks need to be tackled first in order to turn the abovementioned challenges into opportunities. Based on the results of the different studies, practical guidelines and recommendations for farmers, veterinarians and the industry are generated by implementing fixes for veterinarian and his clients through veterinary herd health management programs.

Chapter 3

Udder Health on Flemish Dairy Farms

Chapter 3.1

Management Practices associated with Clinical Mastitis and Bulk Milk Somatic Cell Count on Flemish Dairy Farms

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In preparation

Abstract

Associations between dairy herd management practices and characteristics, collected through a web-based questionnaire from 242 dairy herds in Flanders (Belgium), and their udder health status were studied. Herds were classified into four groups based on their bulk milk somatic cell count (BMSCC) and the estimated incidence of clinical mastitis (EICM). Four separate binary logistic regression models with the likelihood of being a good udder health herd (BMSCC ≤ 250,000 cells/mL and EICM ≤ 3%/month), a high clinical mastitis herd (CM herd) (BMSCC ≤ 250,000 cells/mL and EICM >3%/month),a high subclinical mastitis herd (SCM herd) (BMSCC > 250,000 cells/mL and EICM ≤ 3%/month), and a mixed herd (BMSCC > 250,000 cells/mL and EICM > 3%/month) as dependent variables were fit.

Only one-third (35.1%) of the participating herds had low BMSCC (≤ 250,000 cells/mL) and a low EICM (≤3%/month) and were considered as good udder health herds. Thirty (12.4%) herds were classified as SCM herds, whereas 74 (30.6%) were defined as CM herds. Mixed herds counted for 21.9 % (n = 53) of the participating herds. No strong correlation was found between BMSCC and EICM in this study. Several management practices and characteristics were associated with the likelihood of being a good udder health herd, of being a CM herd, a SCM herd and a mixed herd. Participation in a veterinary herd health monitoring program increased the probability of having a good udder health and decreased the probability of having CM problems. Different management practices were associated with the likelihood of being a CM or SCM herd, suggesting that herds that suffer from SCM require a different approach than the ones that suffer from CM.

Introduction

Despite the fact that much research and effort have been dedicated to mastitis control, it remains a persistent problem and is still the most expensive disease in dairy cows (Schepers and Dijkhuizen, 1991; Lam et al., 2013). The disease can have two very distinctive presentations: subclinical or clinical, depending on both the pathogen involved and the immune response of the cow (Barnouin et al., 2005). Some studies suggested that a low BMSCC is associated with a high incidence rate of clinical mastitis (IRCM) (Elbers et al., 1998; Beaudeau et al., 2002; Green et al., 2004; Riekerink et al., 2008) whereas other work did not reveal any relationship (Barkema et al., 1998a; Beaudeau et al., 1998) between both types of mastitis.

Throughout the world, the importance of udder health programs has increased in the last 30 years. In Europe, the European economic community (EEC) directive 92/46 in April 1992 stated that bulk milk with a SCC over 400,000 cells/mL may not be used for fluid milk and from 1998 on not for milk products intended for human consumption such as cheese. However, as shown by Schukken et al. (1990), annual mean BMSCC should be lower than 250,000 cells/mL to minimize both the risk of exceeding the penalty limit of 400,000 cells/mL in any given bulk tank evaluation and milk production losses due to subclinical mastitis. In Flanders, Belgium, the BMSCC decreased from above 300,000 cells/mL in 1991 to 196,000 cells/mL in 1999. Since that time, however, the geometric mean BMSCC increased again to 214,200 cells/mL in 2014 (Annual Report 2014, Milk Control Centre Flanders, Lier, Belgium).

Increased awareness of consumer and dairy organizations with regard to animal welfare issues is another aspect linked to udder health. Clinical mastitis may be a severe and painful disease that causes distress to the animal. Based on several studies across Europe (Barkema et al, 1998a; Barnouin et al., 2005; Bradley et al., 2007; Wolf et al., 2012) the average IRCM can be expected to be approximately 2.8% cases per month. Recently, Verbeke et al. (2014) estimated the mean IRCM in Flemish dairy herds at 2.4% cases per month and showed a high between-herd variation (range: 0 - 21.3 quarter cases per 10,000 cow-days at risk).

Human health concerns regarding milk consumption represents a third issue related to mastitis. The risk of antibiotic residues in milk, transfer of antibiotic resistance from animal to human, and transfer of pathogens or pathogen products through milk or milk products are of concern to the dairy consuming public. While antibiotic residues in milk are rare in developed industries, approximately 80% of antibiotic residues in milk can be traced back to mastitis treatments, either during lactation of during dry period (Leslie et al., 1997; Ruegg et al., 2000).

Along with the introduction of penalty limits for the BMSCC, the development and widespread acceptance of mastitis prevention and national dairy herd control programs (Neave et al., 1969; National Mastitis Council, 2006) has led to a substantial progress in controlling subclinical mastitis world-wide. Management practices potentially associated with a lower BMSCC have been extensively studied in Belgium (Piepers et al., 2010) and other countries (Neave et al., 1969; Goodger et al., 1993; Barkema et al., 1998a). However, only a few groups have published peer-reviewed research on risk factors for CM (Peeler et al., 2000; Barnouin et al., 2005; Verbeke et al., 2014).

The main objective of the present epidemiological study was to identify, from a large list of potentially relevant variables, dairy herd management practices and characteristics associated with the udder health status on Flemish dairy farms. The udder health status was defined based on a combination of clinical and subclinical mastitis data.

Materials and Methods

Herd Selection and Data Collection

A web-based questionnaire was conducted between January 2010 and July 2010. The questionnaire was pre-tested and refined in close cooperation with 4 dairy farmers prior to the start of the study. The questionnaire was emailed to approximately 1000 farmers based on a list (subscribers on the weekly newsletter) from the largest farmer's organization (Boerenbond) in Flanders and an incentive (USB-stick) for a completed questionnaire was provided.

In total, 242 farmers (almost 25% of those solicited and representing approximately 4% of Flemish farmers) completed the online questionnaire that consisted of 43 questions concerning general management (n = 8), herd health management (n = 9), milking (n = 11), calving and dry cow management (n = 9) and nutrition (n = 6), in place on farm during 2009 (Table 1). The survey tool (in Dutch) is available from the corresponding author.

From all farms that completed the online questionnaire, the BMSCC records measured between January 2009 and January 2010, were retrieved from the Milk Control Centre Flanders that executes the regulatory farm screening program in Flanders. All analyses were conducted on unpasteurized bulk milk samples collected in 30 mL sterile screw-cap tubes by trained milk haulers. The samples were kept cool (± 4°C) until arrival at the laboratory.

Table 1. Overview of all herd management practices collected via a web-based questionnaire on 242 dairy herds in Belgium.

General management	
	Type of livestock farming, expected time the farm will still exist, herd size, number of lactating cows, milk quota size, duration of access to pasture during summer, barn type, cleaning frequency of the housing
Herd health management	
	Registration of animal diseases, herd health monitoring by veterinarian, participation in udder health monitoring, monthly estimated incidence of clinical mastitis, antimicrobial treatment during lactation of subclinical mastitis, treatment of prepartum heifers with antimicrobials, treatment based on vet advice or culture results, treatment duration of mild clinical mastitis, use of self-prepared off-label udder infusions
Milking management	
	Milking machine type, milking parlor type, cows kept in headlock after milking, use of automatic cluster removal, providing a preparation lag-time of 60 s, teat preparation method, application of premilking teat disinfection, application of postmilking teat disinfection, type of machine unit liner, rinsing of machine unit liners, replacement of machine unit liners
Calving management	
	Calving on pasture, presence of calving pen, use of calving pen for sick cows
Nutrition management	
	Concentrate provided during milking, concentrate provided on top of forage, concentrate provided via total mixed ration, concentrate provided via automatic feeder, forage provided, type of forage feeding system
Dry cow management	
	Drying-off procedure, use of long-acting antimicrobials, adapted diet provided, mineral/vitamin mix provided, use of external teat sealer, use of internal teat sealer

Laboratory Analyses

The bulk milk somatic cell count was determined within 24h after pick-up at the farm. The milk samples were vortexed prior to the start of the analyses. Milk SCC was quantified by electronic counting using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) at the Milk Control Centre Flanders (Lier, Belgium). A monthly geometric mean BMSCC was calculated based on the recordings (4 recordings per month) of the last 2 months.

Definitions

Estimated incidence of clinical mastitis (EICM). Clinical mastitis data were collected via the questionnaire. Clinical mastitis was defined as the presence of visual signs such as clots

in the milk, with or without redness, swelling of the udder quarter, or systemic signs (Lam et al., 2009). Farmers were asked to fill in the average number of CM cases per month. The EICM was calculated as the number of average CM cases per month divided by the average number of lactating cows in the herd during 2009 and expressed as a percentage. The threshold for defining an elevated EICM was set at 3% which was based on the average percentage of CM reported accross several European studies (Barkema et al., 1998a; Barnouin et al., 2005; Bradley et al., 2007; Wolff et al., 2012, and Verbeke et al., 2014).

Bulk milk somatic cell count. Per herd, the average of the monthly geometric mean of four measurements per month was calculated. Based on the latter BMSCC value, the herds were classified into two groups using the threshold proposed by Schukken et al. (1990) and others (Erskine and Eberhart, 1991; Barkema et al., 1998a, Rodrigues et al., 2005). Herds with a BMSCC ≥ 250,000 cells/ml were considered as high BMSCC herds while herds with a BMSCC < 250,000 cells/ml were considered as low BMSCC herds.

Udder health status. Herds having a good udder health were defined as those herds where the average monthly EICM was ≤ 3% and the average BMSCC was ≤ 250,000 cells/mL. Herds with an average monthly EICM >3% and an average BMSCC ≤ 250,000 cells/mL were defined as high clinical mastitis herds (further referred as CM herds) . Herds with an average BMSCC > 250,000 cells/mL and an average monthly EICM ≤ 3% were defined as high subclinical mastitis herds (further referred as SCM herds). High subclinical and clinical mastitis herds (further referred as mixed herds) were defined as herds with an average BMSCC > 250,000 cells/mL and an average monthly EICM > 3%.

Statistical Analyses

All data were entered in an electronic spreadsheet program (Excel 2010, Microsoft Corporation) and were checked for unlikely values. Bulk milk somatic cell count and (n = 2616) and EICM were available for 242 farms that fully completed the questionnaire. A natural logarithmic transformation of the BMSCC data was performed in order to obtain a normal distribution (LnBMSCC). Pearson correlation coefficient between EICM and the LnBMSCC was assessed by using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

The regression model-building process to identify management practices potentially associated with the likelihood of being a good udder health herd, a CM herd, a SCM herd, and a mixed herd involved several steps as described previously (De Vliegher et al., 2004). Four separate binary logistic regression models with four different binary dependent variables (good udder health herds versus the other herds; CM herds versus good udder health herds; SCM

herds versus good udder health herds; mixed herds versus good udder health herds) were fit using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

Initially, unconditional associations were tested between the three different dependent herd-level variables and all management practices (n = 41, Table 1). Statistical significance in this step was assessed at $P < 0.15$. Some of the categorical variables were recoded for biological plausibility reasons or because of low frequencies in one or more categories in this step. For example, the five categories describing frequency (never, rarely, sometimes, usually and always) were combined in two categories. Second, Pearson and Spearman's rank correlation coefficients were calculated among the significant independent variables to avoid multicollinearity in the next steps. If two independent variables had a correlation coefficient ≥ 0.6, only the one with the highest statistical significance or the most biologically relevant variable was selected for further analysis. In the third step, multivariable models were built with the remaining management practices as independent variables, and with good udder health, CM herd, SCM herd and mixed herd, respectively, as dependent variables (Table 1). Non-significant variables were removed using backwards elimination at $P \leq 0.05$. In all models, all first order interactions between the remaining variables in the multivariable model were tested and removed when non-significant (Wald's tests, $P > 0.05$).

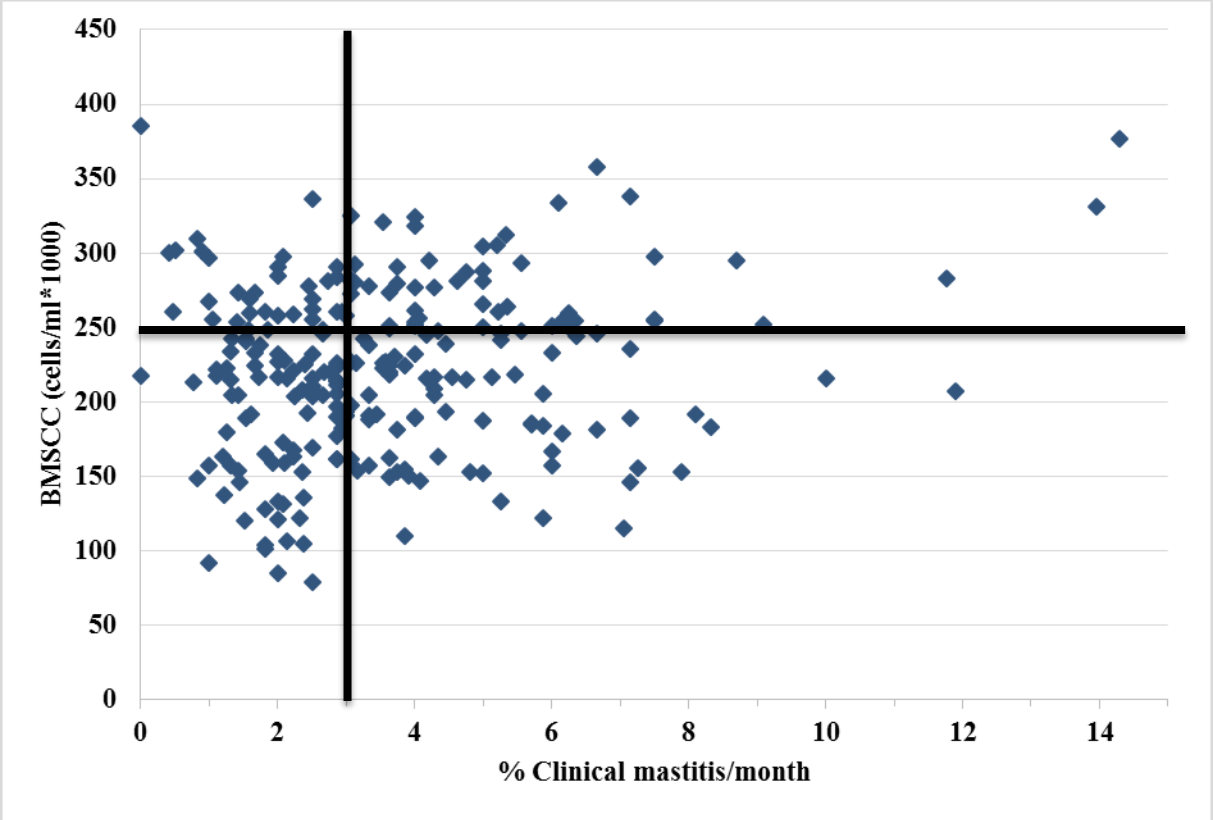

Figure 1. Average bulk milk somatic cell count and estimated incidence of clinical mastitis per month (% clinical mastitis per month) in Flemish dairy herds in 2009.

Results

Descriptive Results

In total, 242 farmers completed the questionnaire. The average herd size of the 218 remaining herds included in the study was 65.8 [interquartile range (IQR) from 45 to 80] lactating cows per herd with an average milk quota size of 552,188 kg of milk (IQR from 375,750 to 676,500) per year. The average of the BMSCC was 202,000 cells/mL (IQR from 155,000 to 239,800 cells/mL) and the median was 224,083 cells/mL. The average monthly EICM was 3.9% (IQR from 2.1% to 4.7%) and the median was 3.1%.

Of the 242 herds, 94.6% (n = 229) used a conventional milking system, whereas 5.4% (n = 13) was milking their cows with an AMS. Eighty percent of the farms (n = 194) housed their cows in a freestall with slatted floor. In almost 60% of the herds (n = 138), some herd health aspects such as fertility, and (or) udder health, and (or) heifer rearing, and (or) claw health were monitored by a veterinarian on a regular basis. Disease recording was systematically done on approximately half of the herds. More than 80% (n = 198) of the farmers expected that their farm would still exist for more than 10 years.

The distribution of BMSCC and EICM in the participating herds in 2009 is shown in Figure 1. One-third (n = 85) of the herds were classified as having a good udder health, whereas 12.4% and 30.6% were classified as SCM herds (n=30) and CM herds (n=74), respectively. Forty-nine herds (21.9%) were classified as mixed herds. The Pearson correlation coefficient between the LnBMSCC and EICM was 0.23 ($P \leq 0.01$).

Unconditional Associations

Risk factors associated ($P \leq 0.15$) with achieving good udder health are listed in Table 2. Risk factors associated ($P \leq 0.15$) with the likelihood of being a CM herd are listed in Table 3. In this model, a strong correlation was found between participation in a veterinary herd health monitoring (VHHM) program and participation in an udder health monitoring program. The latter variable had the highest statistical significance and was therefore selected for further analysis. Table 4 gives an overview of the risk factors associated ($P \leq 0.15$) with the likelihood of being a SCM herd. In this model, a strong correlation was found between the type of milking machine and automated milking system (AMS). The latter variable had the highest statistical significance and was therefore selected for further analysis. Table 5 presents the risk factors associated ($P \leq 0.15$) with the likelihood of being a mixed herd. A strong correlation was again found between participation in a VHHM program and participation in an udder health monitoring program and between the type of milking machine and AMS.

Table 2. Unconditional associations for achieving low bulk milk somatic cell count (≤ 250,000 cells/mL) and low estimated incidence of clinical mastitis (≤ 3%/month) in Flemish dairy herds ($P < 0.15$).

	Good udder health herds				
Independent variables	**Herds (n)**	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
General management & demography					
Province					0.081
Antwerpen	51	Ref.	…		
Limburg	40	-0.026	0.486	0.974	0.957
Oost Vlaanderen	48	0.472	0.441	1.603	0.284
Vlaams Brabant	5	-0.314	1.163	0.731	0.787
West Vlaanderen	98	0.868	0.380	2.382	0.022
Automated milking system					0.144
No	229	Ref.	…		
Yes	13	-1.14	0.781	0.32	
Herd health management					
Participation in veterinary herd health program					0.042
No	104	Ref.	…		
Yes	138	0.569	0.279	1.766	
Participation in udder health monitoring program					0.027
No	111	Ref.	…		
No, but other items are monitored	27	1.173	0.445	3.233	0.008
Yes	104	0.415	0.294	1.515	0.158
Percentage of culling/year	242	-0.038	0.014	0.963	0.008
Treatment decision based on vet advice or culture results					0.074
Never/rarely/sometimes	79	Ref.	…		
Always/usually	163	-0.506	0.283	0.603	
Treatment duration mild clinical mastitis					0.098
Always/usually 3days or more	191	Ref.	…		
Always/usually less than 3days	22	-0.282	0.481	0.751	0.294
No protocol in duration	29	-1.088	0.514	0.337	0.123

Table 2. (continued).

	Good udder health herds				
Independent variables	**Herds (n)**	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
Milking management					
Cows kept in headlocks after milking					0.002
No, although possible	135	Ref.	…		
Yes, but not all animals	28	-0.731	0.527	0.481	0.166
Yes, all animals	68	0.913	0.306	2.491	0.003
Not possible (e.g. tiestall)	11	-0.709	0.804	0.492	0.377
Use of automatic cluster removal					0.048
No	35	Ref.	…		
Yes	207	0.883	0.446	2.419	
Application of postmilking teat disinfection					0.055
Never / rarely / sometimes	24	Ref.	…		
Always / usually	218	1.084	0.565	2.956	
Calving management					
Calving on pasture in 2009					0.064
No	66	Ref.	…		
Yes, some/all	176	0.596	0.321	1.815	
Dry cow management					
Mineral/vitamin mix provided					0.050
No	64	Ref.	…		
Yes	178	0.641	0.327	1.899	
Use of long-acting antimicrobials					0.149
Blanket	222	Ref.	…		
No	0				
Selective	20	0.832	0.576	2.298	
Drying-off procedure					0.109
Abrupt	172	Ref.	…		
Intermittent milking	70	0.467	0.292	1.595	

[1]SE = Standard error, [2]OR = Odds ratio.

Table 3. Unconditional associations for a high estimated incidence of clinical mastitis (> 3%/month) and low bulk milk somatic cell count (≤ 250,000 cells/mL) in Flemish dairy herds ($P < 0.15$).

	Clinical mastitis herds				
Independent variables	**Herds (n)**	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
Herd health management					
Participation in veterinary herd health program					0.029
No	67	Ref.	…		
Yes	92	-0.712	0.326	0.491	
Participation in udder health monitoring program					0.069
No	67	Ref.	…		
No, but other items are monitored	22	-0.617	0.346	0.540	0.075
Yes	70	-1.032	0.520	0.356	0.047
Percentage of culling/year	159	0.049	0.018	1.050	0.008
Treatment decision based on vet advice or culture results					0.005
Never/rarely/sometimes	48	Ref.	…		
Always/usually	111	1.050	0.370	2.857	
Sensitivity testing performed in case of culturing					0.042
Never/rarely/sometimes	131	Ref.	…		
Always/usually	28	0.880	0.432	2.411	
Dry cow management					
Use of internal teat sealer					0.088
No	96	Ref.	…		
Only high cell count cows	13	-0.474	0.636	0.622	0.455
All cows	45	0.835	0.371	2.306	0.024
Only low cell count cows	5	-0.069	0.936	0.933	0.941

[1]SE = Standard error, [2]OR = Odds ratio.

Table 4. Unconditional associations for high bulk milk somatic cell count (> 250,000 cells/mL) and low estimated incidence of clinical mastitis (≤ 3%/month) in Flemish dairy herds ($P < 0.15$).

	Subclinical mastitis herds				
Independent variables	**Herds (n)**	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
General management & demography					
Number of lactating cows	115	0.013	0.007	1.012	0.051
Percentage of culling/year	115	0.023	0.015	1.023	0.140
Herd health management					
Cows kept in headlocks after milking					0.015
No, although possible	62	Ref.	…		
Yes, but not all animals	11	0.924	0.661	2.520	0.164
Yes, all animals	39	-1.743	0.659	0.175	0.008
Not possible (e.g. tiestall)	3	0.049	1.255	1.050	0.969
Milking management					
Automatic milking system					0.015
No	108	Ref.	…		
Yes	7	2.116	0.867	8.3	
Milking machine type					0.108
Low line set-up	88	Ref.	…		
High line set-up	5	-0.163	1.147	0.850	0.887
With glass jars	15	0.212	0.637	1.236	0.739
Automated milking system	7	2.14	0.874	8.5	0.140
Application of the 60sec rule for lag time					0.140
Never / rarely / sometimes	49	Ref.	…		
Not possible	9	1.349	0.749	3.854	0.071
Always / usually	57	-0.093	0.458	0.911	0.834
Postmilking teat disinfection					0.048
Never / rarely / sometimes	9	Ref.	…		
Always / usually	106	-1.399	0.709	0.247	

Table 4. (continued).

Independent variables	Subclinical mastitis herds				
	Herds (n)	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Calving management					
Did cows calve on pasture in 2009					0.002
No	32	Ref.	…		
Yes, some/all	83	-1.386	0.455	0.250	
Nutrition management					
Access to pasture during summer of 2009					0.138
No	12	Ref.	…		
Yes, but limited	75	-1.306	0.657	0.271	0.047
Yes, more than 12hours/day	28	-1.036	0.676	0.355	0.125
Dry cow management					
Mineral/vitamin mix provided					0.010
No	29	Ref.	…		
Yes	86	-1.193	0.461	0.303	
Drying-off procedure					0.063
Abrupt	80	Ref.	…		
Intermittent milking	35	-1.003	0.540	0.367	
Use of long-acting antimicrobials					0.126
Blanket	107	Ref.	…		
Selective	8	1.136	0.742	3.115	

[1]SE = Standard error, [2]OR = Odds ratio.

Table 5. Unconditional associations for achieving high bulk milk somatic cell count (> 250,000 cells/mL) and high estimated incidence of clinical mastitis (> 3%/month) in Flemish dairy herds (*P* < 0.15).

	Mixed herds				
Independent variables	**Herds (n)**	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
General management & demography					
Province					0.147
Antwerpen	25	Ref.	…		
Limburg	23	0.342	0.581	1.408	0.555
Oost Vlaanderen	28	-0.355	0.557	0.701	0.523
Vlaams Brabant	1	-21.123	40192	0	1
West Vlaanderen	61	-0.871	0.492	0.419	0.077
Automated milking system					0.021
No	131	Ref.	…		
Yes	7	1.464	0.856	4.323	
Herd health management					
Participation in veterinary herd health program					0.128
No	54	Ref.	…		
Yes	84	-0.545	0.358	0.580	
Participation in udder health monitoring program					0.100
No	54	Ref.	…		
No, but other items were monitored	18	-1.461	0.689	0.232	0.034
Yes	66	-0.346	0.373	0.353	0.707
Percentage of culling/year	138	0.046	0.019	1.048	0.017
Person who is treating a clinical mastitis cases					0.104
Farmer	66	Ref.	…		
Veterinarian (always or in specific cases)	72	-0.574	0.354	0.563	
Treatment duration mild clinical mastitis					0.052
Always/usually 3 days or more	110	Ref.	…		
Always/usually less than 3days	13	0.525	0.592	1.691	0.375
No fixed treatment duration	15	1.373	0.584	3.946	0.019

Table 5. (continued).

	Mixed herds				
Independent variables	**Herds (n)**	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Use of local and parenteral antimicrobials when clinical mastitis					0.071
Never/rarely/sometimes	68	Ref.	…		
Always/usually	55	-0.687	0.380	0.503	
Use of self-prepared off-label udder infusion					0.128
Never	49	Ref.	…		
Always/usually	89	-0.544	0.364	0.575	
Milking management					
Cows kept in headlocks after milking					0.011
No, although possible	74	Ref.	…		
Yes, but not all animals	13	0.742	0.616	2.100	0.229
Yes, all animals	45	-1.114	0.440	0.328	0.011
Not possible (e.g. tiestall)	6	0.965	0.897	2.625	0.282
Use of automatic cluster removal					0.021
No	19	Ref.	…		
Yes	119	-1.182	0.513	0.307	
Milking machine type					0.029
Low line	88	Ref.	…		
High line	5	1.565	0.638	4.781	0.014
With glass jars	15	0.302	0.529	1.352	0.568
Automated milking system	7	1.670	0.864	5.312	0.053
Providing a preparation lag time of 60 s					0.053
Never / rarely / sometimes	50	Ref.	…		
Not possible	10	1.451	0.722	4.269	0.044
Always / usually	78	0.788	0.395	2.199	0.046
Type of the machine unit liners					0.133
Rubber	123	Ref.	…		
Silicone	15	-1.008	0.671	0.365	

Table 5. (continued).

	Mixed herds				
Independent variables	**Herds (n)**	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
Postmilking teat disinfection					0.045
Never / rarely / sometimes	12	Ref.	…		
Always / usually	126	-1.281	0.640	0.278	
Calving management					
Calving on pasture in 2009					0.112
No	34	Ref.	…		
Yes, some/all	104	-0.636	0.400	0.529	
Presence of calving pen					0.015
No (tiestall/cubicles)	27	Ref.	…		
Yes	111	-1.068	0.440	0.344	
Calving pen used of sick pen					0.029
No calving pen	21	Ref.	…		
Yes	74	-1.346	0.516	0.260	0.009
No	43	-0.814	0.545	0.443	0.136
Dry cow management					
Mineral/vitamin mix provided					0.008
No	37	Ref.	…		
Yes	101	-1.040	0.395	0.353	
Adapted diet provided					0.126
No	10	Ref.	…		
Yes	128	-0.682	0.446	0.506	

[1]SE = Standard error, [2]OR = Odds ratio.

Based on the statistical significance, participation in an udder health monitoring program and type of milking machine were selected for further analysis.

Final Multivariable Models

The final multivariable models are presented in Tables 6, 7, 8 and 9. In none of the multivariable models, any of the tested interaction terms was significant ($P < 0.05$).

Risk factors associated with achieving good udder health (Table 6). The final model revealed that herds where all cows are fixed in the headlocks after milking, the likelihood of having good udder health was 2.4 times higher than on farms where cows were not fixed in the headlocks ($P = 0.007$). The culling rate was positively associated with the likelihood of achieving a good udder health ($P = 0.003$). On herds that participated in a VHHM program that focused on other herd health items than udder health (e.g. reproduction), the likelihood of achieving a good udder health was 3.5 times higher than on herds that didn't participate in any udder health monitoring ($P = 0.009$).

Risk factors associated with CM herds (Table 7). Herds that did not participate in a VHHM program were significantly more likely to be classified as a CM herd (P = 0.004). The likelihood of having a high CM increased with an increasing culling rate (P = 0.015). Also, herds where the treatment decisions were usually or always based on the advice of the veterinarian or based on culture results, were significantly more likely to be classified as a CM herd (P = 0.012). When all cows are dried off with an internal teat sealer, the likelihood of being a CM herd was 3.1 times higher than when no internal teat sealers were used ($P = 0.007$).

Risk factors associated with SCM herds (Table 8). Postmilking teat disinfection (PMTD) ($P = 0.018$) and feeding dry cow minerals significantly decreased the odds of being classified as a SCM herd ($P = 0.030$). Also, herds with no access to pasture in summer ($P = 0.027$) and herds where cows were not calving at pasture were more likely to be associated with a SCM herds ($P = 0.010$). Keeping cows in the headlocks after milking ($P = 0.020$) and intermittent milking before dry-off cows ($P = 0.009$), were decreasing the odds for being classified as a SCM herd. On the contrary, milking cows with an AMS ($P = 0.019$) and selective dry cow therapy with long-acting antimicrobials ($P = 0.037$), were significantly increasing the odds for being a SCM herd.

Risk factors associated with mixed herds (Table 9). Application of postmilking teat disinfection (PMTD) ($P = 0.019$) and providing a mineral/vitamin mix significantly decreased the odds of being classified as a mixed herd (P = 0.025). Also, herds with a high line milking machine ($P = 0.005$) and herds where cows did not calve at pasture were more likely to suffer

from both SCM and CM (P = 0.047). The likelihood of being a mixed herd increased with an increasing culling rate (P = 0.021). The use of self-prepared off-label udder infusions (P = 0.042) and keeping cows in the headlocks after milking (P = 0.037), significantly decreased the odds of being a mixed herd. Providing a lag preparation time of 60s increased the likelihood of being a mixed herd almost 4 times (P = 0.013) compared to herds where no lag time of 60 s was provided.

Table 6. Final multivariable model for low bulk milk somatic cell count (≤ 250,000 cells/mL) and low estimated incidence of clinical mastitis (≤ 3%/month) in Flemish dairy herds (*P* < 0.05).

	Good udder health			
Independent variables	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Participation in udder health monitoring program				0.034
No	Ref.	…		
Yes	0.300	0.314	1.350	0.340
No, but other items were monitored	1.256	0.483	3.512	0.009
Percentage of culling/year	-0.045	0.015	0.956	0.003
Cows kept in headlocks after milking				0.004
No, although possible	Ref.	…		
Yes, but not all animals	-0.699	0.543	0.497	0.199
Yes, all animals	0.869	0.322	2.385	0.007
Not possible (e.g. tiestall)	-1.049	0.842	0.350	0.213

[1]SE = Standard error, [2]OR = Odds ratio.

Table 7. Final multivariable model for high estimated incidence of clinical mastitis (> 3%/month) and low bulk milk somatic cell count (≤ 250,000 cells/mL) in Flemish dairy herds ($P < 0.05$).

	Clinical mastitis herds			
Independent variables	**Beta**	**SE[1]**	**OR[2]**	**P value**
Participation in veterinary herd health program				0.004
No	Ref.	…		
Yes	-1.091	0.376	0.336	
Percentage of culling/year	0.048	0.020	1.049	0.015
Treatment decision based on vet advice or culture results				0.012
Never/rarely/sometimes	Ref.	…		
Always/usually	1.007	0.399	2.737	
Use of internal teat sealer				0.026
No	Ref.	…		
Only high cell count cows	-0.643	0.680	0.525	0.344
All cows	1.122	0.413	3.072	0.007
Only low cell count cows	0.150	0.991	1.161	0.880

[1]SE = Standard error, [2]OR = Odds ratio.

Table 8. Final multivariable model for high bulk milk somatic cell count (> 250,000 cells/mL) and low estimated incidence of clinical mastitis (≤ 3%/month) in Flemish dairy herds (*P* < 0.05).

	Subclinical mastitis herds			
Independent variables	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Cows kept in headlocks after milking				0.010
No, although possible	Ref.	…		
Yes, but not all animals	1.728	0.851	5.629	0.042
Yes, all animals	-2.007	0.863	0.134	0.020
Not possible (e.g. tiestall)	-2.616	2.402	0.073	0.276
Automatic milking system				0.019
No	Ref.	…		
Yes	2.550	1.084	12.805	
Application of postmilking teat disinfection				0.018
Never / rarely / sometimes	Ref.	…		
Always / usually	-2.261	0.953	0.104	
Calving on pasture in 2009				0.010
No	Ref.	…		
Yes, some/all	-1.644	0.635	0.193	
Access to pasture during summer of 2009				0.027
No	Ref.	…		
Yes, but limited	-1.865	0.910	0.155	0.040
Yes, more than 12hours/day	-0.136	0.930	0.873	0.884
Mineral/vitamin mix provided				0.030
No	Ref.	…		
Yes	-1.337	0.616	0.263	
Drying-off procedure				0.009
Abrupt	Ref.	…		
Intermittent milking	-1.925	0.736	0.146	
Use of long-acting antimicrobials				0.037
Blanket	Ref.	…		
Selective	2.148	1.032	8.570	

[1]SE = Standard error, [2]OR = Odds ratio.

Table 9. Final multivariable model for high bulk milk somatic cell count (> 250,000 cells/mL) and high estimated incidence of clinical mastitis (> 3%/month) in Flemish dairy herds ($P < 0.05$).

	Mixed herds			
Independent variables	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Percentage of culling/year	0.056	0.024	1.058	0.021
Use of self-prepared off-label udder infusion				0.042
Never	Ref.	…		
Always/usually	-1.005	0.494	0.366	
Cows kept in headlocks after milking				0.027
No, although possible	Ref.	…		
Yes, but not all animals	1.307	0.739	3.696	0.077
Yes, all animals	-1.134	0.542	0.322	0.037
Not possible (e.g. tiestall)	-1.190	1.510	0.304	0.431
Milking machine type				0.014
Low line	Ref.	…		
High line	2.959	1.054	19.275	0.005
With glass jars	-0.230	0.721	0.794	0.749
Automated milking system	3.425	1.907	30.725	0.072
Providing a preparation lag time of 60 s				0.022
Never / rarely / sometimes	Ref.	…		
Not possible	-1.235	1.722	0.291	0.473
Always / usually	1.383	0.556	3.988	0.013
Application of postmilking teat disinfection				0.019
Never / rarely / sometimes	Ref.	…		
Always / usually	-1.869	0.799	0.154	
Calving on pasture in 2009				0.047
No	Ref.	…		
Yes, some/all	-1.055	0.531	0.348	
Mineral/vitamin mix provided				0.025
No	Ref.	…		
Yes	-1.103	0.493	0.332	

[1]SE = Standard error, [2]OR = Odds ratio.

Discussion

This is the first study that focused on the identification of risk factors for udder health on Flemish dairy herds in which the udder health status was defined based on BMSCC data combined with CM data, two primary parameters for udder health (Barkema et al., 2013). To determine which management factors were associated with CM and BMSCC in Flanders, a web-based questionnaire was used. The latter way of working allowed, for a relatively easy, efficient and cost-effective data collection. The response rate was around 24%, which represented 4% of the dairy farmers in Flanders in 2009 (Annual Report 2009, Belgian Dairy Confederation, Leuven, Belgium). The average herd and quota size (65.8 lactating cows and 552,188 kg of milk per year) of the selected farms were higher than the Flemish average, being 44 lactating cows and 310,650 kg of milk per herd, respectively (Annual Report 2009, Belgian Dairy Confederation, Leuven, Belgium). Herd size and the need to have internet access, might have selected the input of more contemporary farmers potentially diminishing the external validity of the data (Dohoo et al., 2009). Still, the high variation in BMSCC between the respondents suggests a wide diversity of management styles (Barkema et al., 1998a). The average BMSCC (202,000 cells/mL) of the selected herds was lower than the average BMSCC for the whole of Flanders being 230,000 cells/mL in 2009 (Annual Report 2009, Milk Control Center Flanders, Lier, Belgium). The average EICM was higher than the IRCM found by Verbeke et al. (2014) and other studies from the Netherlands, Sweden and France (Barkema et al., 1998a; Wolff et al., 2012, Barnouin et al., 2005), but lower than the averages found in England and Wales (Bradley et al., 2007). Because of differences in methodology and selection criteria between studies, caution is required in comparing results between CM studies (Olde Riekerink et al., 2008). In our study, the majority of the farmers admitted they were not keeping accurate disease records whereas in the study of Verbeke et al. (2014) farmers agreed to record every CM case and collect a milk sample before treatment. The discrepancy between the results obtained in the latter study and ours contrasts the findings of Peeler et al. (2000) who found that farmers that kept records of CM reported a significantly higher level of mastitis compared with those not keeping records. Also, even if all farmers received the same instructions on the definition of a case of CM, several factors can cause differences in the identification of the cases of CM among farmers including the motivation of the farmer, the skills of the farmer to recognize a clinical case, and differences in management practices (i.e. stripping the foremilk).

Since esculin-positive cocci are the most commonly isolated organisms in both subclinical and clinical mastitis cases in Flanders (Annual Report 2009, Milk Control Centre Flanders, Lier, Belgium), a strong association between EICM and BMSCC was expected, but was not

observed in this study. The lack of a strong relationship between BMSCC and EICM is consistent with the results of some previous research (Beaudeau et al., 1998; Barkema et al., 1998a, Olde Riekerink et al., 2008), but not with the findings of others who reported a strong association between both parameters (Erskine et al., 1988; Elbers et al., 1998; Beaudeau et al., 2002; Green et al., 2004). Since no milk samples for bacteriological culturing were collected in this study, a potential association between BMSCC and the pathogen-specific IRCM as observed by Barkema et al. (1999) and Olde Riekerink et al. (2008) could not be further elaborated. We should also bear in mind that BMSCC is determined on the milk of all lactating cows whose milk is not discarded, making non-saleable milk (e.g. milk from treated cows, milk from cows with a high SCC) an unknown component in the analysis.

Although statistically significant associations were found between management practices and the four types of herds (good udder health, SCM herds, CM herds and mixed herds), those management practices were not necessarily causally related. The only way to determine whether a variable that has a significant association really has a causal relationship is to perform a prospective intervention study as has been done for postmilking teat disinfection (Lam et al., 1997). Also, several factors presented very little variation in the dataset, and failure to observe relationships under these circumstances does not necessarily imply that there is no association between the studied management practices and the outcome variable(s). Some of the management practices were highly adopted in our population of herds. Postmilking teat disinfection and replacing the machine unit liners according to the recommended schedule were for example done by more than 90% and less than 10% of the herds, respectively, making it difficult to demonstrate statistical significance in the multivariable analyses. Shewe et al. (2015) showed that ensuring strict compliance with milking protocols was negatively associated with BMSCC in farms with nonfamily employees. Based on our study design and the type of questions, we were obviously not able to measure compliance of management practices, making us more cautious in interpreting our findings. Jansen et al. (2009) showed that the farmer's attitudes towards mastitis was significantly associated with increased BMSCC and incidence of CM. Since we used the average BMSCC and EICM on a year-base, we could not examine the seasonal effect of some possible management practices. Barkema et al. (1998a) found conflicting significant associations from one season to another for herds keeping their cows standing after milking.

Herds where cows calved at pasture were less likely to be categorized as mixed herds. As almost 50% of the dairy herds in Flanders use their calving pen for both sick and periparturient cows (De Vliegher et al., 2004), we hypothesize that a prolonged stay in a more infectious environment at least partly explains this finding. Herds where cows calved in the barn were

more likely to be SCM herds. The latter was in contrast with the findings of Wenz et al., (2007), who reported that herds without an outside area for peripartum cows were more likely to have a low BMSCC. Access to pasture in summer decreased the likelihood of being categorized as a SCM herd compared to zero-grazing. However, limited access to pasture lowered the odds of being a SCM herd compared to having access more than 12 hours/day. The difference between the two risks factors is probably more reflecting the farmers' attitude concerning cow comfort and nutrition than the difference in infection pressure between inside housing and pasture.

The finding that postmilking teat disinfection and providing a mineral/vitamin mix significantly decreased the odds of being classified as a SCM and mixed herd, was not surprising, despite the fact that very few herds were not applying postmilking teat disinfection. Several research groups have shown that postmilking teat disinfection was associated with a low BMSCC (Erksine et al., 1987; Erskine and Ebelhart, 1991; Hutton et al., 1991; Barkema et al., 1998a; Bareille et al., 1998 and Khaitsa et al., 2000). A similar association was found for providing a mineral/vitamin mix to dry cows and low BMSCC (Erskine et al. (1987); Weiss et al., (1990) and Barkema et al., 1998a).

Herds where the treatment decision for mastitis is usually or always based on the advice of a veterinarian or on culture results are significantly more likely to be classified as a CM herd. Also, herds where all cows were dried off with an internal teat sealer were more likely to suffer from CM. A cause-effect reversal is suspected. Both risk factors are most likely the consequence of dealing with CM, instead of a cause. The same reasoning can be assumed for the findings that self-prepared off-label udder infusions and providing a preparation lag time of 60 s were more often applied on a herd that suffered from both SCM and CM than on a herd classified as having a good udder health. However, the high percentage (66%) of herds that is using self-prepared off-label udder infusions to treat CM is worrying, especially in the light of prudent drug use and antimicrobial resistance. The use of an internal teat sealer was positively associated with the likelihood of being a CM herd. This finding is probably also a cause-effect reversal, since Verbeke et al. (2014) found that a majority of CM cases was found in the beginning of lactation and Bradley et al. (2004) also showed that 60% of those cases originated from the dry period. Finding these cause-effect reversals, could also be interpreted as farmers only change their management practices when problems occur. This latter shows opportunities to a more proactive approach of udder health management.

The fact that milking cows with an AMS is increasing the odds of being a SCM herd corresponds well with the findings from other studies (reviewed by Hovinen and Pyörälä, 2011). Still, this finding stresses the importance of implementing a pro-active mastitis

prevention and control plan on AMS herds. On the other hand, manufacturers of AMS should also be aware of the fact that most of the AMS are less effective in cleaning teats (Knappstein et al., 2004; Bade et al., 2008) and are not able to distinguish and separate abnormal milk (Hovinen et al., 2009).

A higher culling rate increased the likelihood of being a CM herd. This is consistent with the studies done by Peeler et al. (2000) and Barnouin et al. (2005) in low somatic cell count herds, where a higher culling rate was also associated with a higher IRCM. Also, herds with a higher culling rate were significantly less likely to have good udder health and more likely to be classified as a mixed herd. A high culling rate is generally an indication of poor fertility management, which might also result in high levels of mastitis, because farmers are not able to cull cows for other reasons than fertility (e.g. high cell count) to achieve their production goals. In addition, introducing new cows into an established herd may itself be a risk for mastitis (Peeler et al., 2000).

Herds that did not participate in a VHHM program were significantly more likely to be classified as a CM herd. Regular visits of the herd veterinarian in the barn could possibly help to confront farmers with known risk factors for CM which can easily be evaluated during the visit (e.g. hygiene, housing, nutrition).

Also, herds that participated in a monitoring program that focused on other herd health items than udder health (e.g. reproduction), had more chance to be classified as having good udder health. First, farmers that chose to monitor reproduction in their farms, will possibly have a different attitude and have a more intrinsic motivation compared with those that do not, which might be extrapolated to the compliance of their daily (udder health) management. Also, following VHHM programs probably will help farmers to keep better records (e.g. expected calving date) and making the dry period better manageable since for example dry period lengths will have less variations. Fourth, on those herds, veterinarians might have advised the farmers to get rid of their sick or problematic cows (e.g. high cell count cows); they might have recommended to cull some cows where farmers would otherwise maybe have waited, as shown by Derks et al. (2014). We should, however, be cautious in drawing conclusions as the reasons for culling are not known in our study. Still, literature shows that subfertility is generally the most common reason for culling on dairy herds (Bascom et al., 1998 and Ahlman et al., 2010).

Conclusions

Only one-third of the herds in Flanders have low BMSCC (≤ 250,000 cells/mL) and a low EICM (≤ 3%). No strong association between BMSCC and EICM could be found in this study. The latter along with the fact that different management practices were associated with the likelihood of being a SCM or CM herd suggests that both types of herds require a different approach. Several factors are found to be associated with good udder health, CM herds, SCM herds and SCM+CM herds. Participation in a VHHM program seems to have an added value in prevention of udder health problems on Flemish dairy herds.

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References

Ahlman, T., B. Berglund, L. Rydhmer, and E. Strandberg. 2010. Culling Reasons in organic and conventional dairy herds and genotype by environment interaction and longevity. J. Dairy Sci. 94:1568-1575.

Bade, R. D., D. J. Reinemann, and P. D. Thompson. 2008. Robot versus human pre-milking teat preparation. Pages 184-185 in NMC Annual Meeting Proc., New Orleans, LA. National Mastitis Council, Madison, WI.

Bareille, N., H. Seegers, C. Fourichon, F. Beaudeau, and X. Mahler. 1998. Surveneu et expression des Mammites Cliniques et Subcliniques en troupeaux bovins laitiers: Facteurs de risqué liés à la conception et à l'utilisation du bâtiment. Pages 297-300 in Rencontres Autour des Recherches sur les Ruminants. Institut de l'élevage, Paris. France.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, G. Benedictus, and A. Brand. 1998a. Management practices associated with low, medium, and high somatic cell counts in bulk milk. J. Dairy Sci. 81:1917-1927.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998b. Incidence of Clinical Mastitis in Dairy Herds grouped in three categories by Bulk Milk Somatic Cell Count. J. Dairy Sci. 81:411-419.

Barkema, H. W., S. De Vliegher, S. Piepers, and R. N. Zadoks. 2013. Herd level approach to high bulk milk somatic cell count problems in dairy cattle. Veterinary Quarterly, 33(2):82-93.

Barnouin, J., S. Bord, S. Bazin, and M. Chassagne. 2005. Dairy Management Practices Associated with Incidence Rate of Clinical Mastitis in Low Somatic Cell Score Herds in France. J. Dairy Sci. 88:3700-3709.

Bascom, S. S., and A. J. Young. 1998. A Summary of the Reasons why Farmers Cull Cows. J. Dairy Sci. 81:2299-2305.

Bradley, A. J., and M. J. Green. 2004. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. Vet. Clin. North Am. Food Anim. Pract. 20:547–568.

Beaudeau, F., H. Seegers, C. Fourichon, and P. Hortet. 1998. Association between milk somatic cell counts up to 400,000 cells/mL and clinical mastitis in French Holstein cows. Vet. Rec. 143:685-687.

Beaudeau, F., C. Fourichon, H. Seegers, and N. Bareille. 2002. Risk of clinical mastitis in dairy herds with a high proportion of low individual milk somatic cell-count. Prev. Vet. Med. 53:43-54.

Belgian Dairy Confederation. 2009. Annual report. Belgian Dairy Confederation, Leuven, Belgium.

Bradley, A. J., K. A. Leach, J. E. Breen, L. E. Green, and M. J. Green. 2007. Survey of the Incidence and Aetiology of Mastitis on Dairy Farms in England and Wales. Vet. Rec. 160:253-258.

De Vliegher, S., H. Laevens, H. W. Barkema, I. R. Dohoo, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Management Practices and Heifer characteristics associated with early lactation Somatic Cell Count of Belgian Dairy Heifers. J. Dairy Sci. 87:937-947.

Derks, M., T. van Werven, H. Hogeveen, and W. D. J. Kremer. 2013. Associations between Farmer participation in Veterinary Herd Health Management Programs and Farm Performance. J. Dairy Sci. 97:1336-1347.

Dohoo, I., M. Wayne, and H. Stryhn (Eds.). 2009. Questionnaire design. In: Veterinary Epidemiologic Research 2nd Edition, VER Incorporated, Charlottetown, Canada p. 58-72.

Elbers, A. R. W., J. D. Miltenburg, D. De Lange, A. P. P. Crauwels, H. W. Barkema, and Y. H. Schukken. 1998. Risk factors for clinical mastitis in a random sample of dairy herds from the southern part of The Netherlands. J. Dairy Sci. 81:420-426.

Erskine, R. J. , R. J. Eberhart, L. J. Hutchinson, S. B. Spencer, and M. A. Campbell. 1987. Herd Management and Prevalence of Mastitis in Dairy Herds with High and Low Somatic Cell Counts. JAVMA 190:1411-1416.

Erskine, R. J. , R. J. Eberhart, L. J. Hutchinson, S. B. Spencer, and M. A. Campbell. 1988. Incidence and types of Clinical Mastitis in Dairy Herds with High and Low Somatic Cell Counts. J. Am. Vet. Med. Assoc. 192:761-765.

Erskine, R. J. , and R. J. Eberhart. 1991. Post-Milking Teat Dip use in Dairy Herds with High or Low Somatic Cell Counts. J. Am. Vet. Med. Assoc. 199:1734-1736.

Goodger, W. J., T. Ferver, J. Pelletier, P. Johnson, G. de Snayer, and J. Galland. 1993. The association of Milking Management Practices with Bulk Tank Somatic Cell Counts. Prev. Vet. Med. 15:235-251.

Green, M. J., P. R. Burton, L. E. Green, Y. H. Schukken, A. J. Bradley, E. J Peeler, and G. F. Medley. 2004. The use of Markov Chain Monte Carlo for analysis of correlated binary data: Patterns of Somatic Cells in Milk and the Risk of Clinical Mastitis in Dairy Cows. Prev. Vet. Med. 64:157-174.

Hovinen, M., M. D. Rasmussen,and S. Pyörälä. 2009. Udder health of cows changing from tie stall or free stall with conventional milking to free stall with either conventional or automatic milking. J. Dairy Sci. 92: 3696–3703.

Hovinen, M., and S. Pyörälä. 2011. Invited review: Udder health of dairy cows in automatic milking. J. Dairy Sci. 94:547-562.

Hutton, C. T., L. K. Fox, and D. D. Hancock. 1990. Mastitis Control Practices: Differences between High and Low Milk Somatic Cell Counts. J. Dairy Sci. 73:1136-1143.

Jansen, J., B. H. P. van den Borne, R. J. Renes, G. van Schaik, T. J. G. M. Lam, and C. Leeuwis. 2009. Explaining Mastitis Incidence in Dutch Dairy Farming: The influence of Farmers' Attitudes and Behaviour. Prev. Vet. Med. 92:210-223.

Khaitsa, M. L., T. E. Wittum, K. L. Smith, J. L. Henderson, and K. H. Hoblet. 2000. Herd Characteristics and Management Practices associated with Bulk-tank Somatic Cell Counts in Herds in official Dairy Herd Improvement association programs in Ohio. Am. J. Vet. Res. 61:1092-1098.

Knappstein, K., N. Roth, H.-G. Walte, J. Reichmuth, B. A. Slaghuis, R. T. Ferwerda-van Zonneveld, and A. Mooiweer. 2004. Effectiveness of automatic cleaning of udder and teats and effects of hygiene management: Report on effectiveness of cleaning procedures applied in different automatic milking systems. Deliverable D14.

Lam, T. J. G. M., J. H. van Vliet, Y. H. Schukken, F. J. Grommers, A. van Velden-Russcher, H. W. Barkema, and A. Brand. 1997. The Effect of discontinuation of Postmilking Teat Disinfection in Low Somatic Cell Count Herds II. Incidence of Clinical Mastitis. Vet. Quart. 19:41-47.

Lam, T. J. G. M., R. G. M. Olde Riekerink, O. C. Sampimon, and H. Smith. 2009. Mastitis diagnostics and performance monitoring: a practical approach. Irish Vet Journal 62:34-39.

Lam, T.J.G.M., B.H.P. Van Den Borne, J. Jansen, K. Huijps, J.C.L. Van Veersen, G. van Schaik, and H. Hogeveen. Improving bovine udder health: A national control program in the Netherlands. J. Dairy Sci. 2013;96:1301–1311.

Leslie, K. E., Y. H. Schukken, J. Sargeant, and M. Mitchell. 1997. Inhibitor Violations during 10 years in Ontario. Association with Somatic Cell Count Reduction Program. Report to the Ontario Buro of Veterinary Drugs.

Milk Control Center Flanders. 2009. Annual Report. Milk Control Center Flanders, Lier, Belgium.

Milk Control Center Flanders. 2014. Annual Report. Milk Control Center Flanders, Lier, Belgium.

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.

Olde Riekerink, R. G. M., H. W. Barkema, D. F. Kelton, and D. T. Scholl. 2007. Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. 2007. J. Dairy Sci. 91:1366-1377.

Peeler, E. J., M. J. Green, J. L. Fitzpatrick, K. L. Morgan, and L. E. Green. 2000. Risk Factors associated with Clinical Mastitis in Low Somatic Cell Count British Dairy Herds. J. Dairy Sci. 83:2464-2472

Piepers. S., H. Van Brempt, K. Supré, P. Passchyn, and S. De Vliegher. 2010. Implementatie van Mastitispreventie-en Controlemaatregelen op Vlaamse Melkveebedrijven. Tijdschr. Diergeneeskd. 135:46-51.

Rodrigues, A. D., D. Z. Caraviello, and P. L. Ruegg. 2005. Management of Wisconsin Dairy Herds enrolled in Milk Quality Teams. J. Dairy Sci. 88:2660-2671.

Ruegg, P. L., and T. J. Tabone. 2000. The relationship between Antibiotic Residue Violations and Somatic Cell Counts in Wisconsin Dairy Herds. J. Dairy Sci. 83:2805-2809.

Schepers, J. A., and A. A. Dijkhuizen. 1991. The economics of Mastitis and Mastitis Control in Dairy Cattle: A critical analysis of estimates published since 1970. Prev. Vet. Med. 10:213-234.

Schewe, R. L., J. Kayitsinga, G. A. Contreras, C. Odom, W. A. Coats, P. Durst, E. P. Hovingh, R. O. Martinez, R. Mobley, S. Moore, and R. J. Erskine. 2015. Herd Management and Social Variables associated with Bulk Tank Somatic Cell Count in Dairy Herds in the eastern United States. J. Dairy Sci. 98:1-16.

Schukken, Y. H., F. J. Grommers, D. van de Geer, and A. Brand. 1989. Incidence of Clinical Mastitis on farms with Low Somatic Cell Counts in Bulk Milk. Vet. Rec. 125:60-63.

Schukken, Y. H., F. J. Grommers, D. van de Geer, 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. 73:3463-3471.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific Incidence Rate of Clinical Mastitis in Flemish Dairy Herds, Severity, and association with Herd Hygiene. J. Dairy Sci. 97:6926-6934.

Weiss, W. P., J. S. Hogan, K. L. Smith, and K. H. Hoblet. 1990. Relationships among Selenium, Vitamine E, and Mammary Gland Health in Commercial Dairy Herds. J. Dairy Sci. 73:381-390.

Wenz, J. R., S. M. Jensen, J. E. Lombard, B. A. Wagner, and R. P. Dinsmore. 2007. Herd Management Practices and their association with Bulk Tank Somatic Cell Count on United States Dairy Operations. J. Dairy Sci. 90:3652-3659.

Wolff, C., M. Espetvedt, A. K. Lind. S. Rintakoski, A. Egenvall, A. Lindberg, and U. Emanuelson. 2012. Completeness of the disease Recording Systems for Dairy Cows in Denmark, Finland, Norway and Sweden with special reference to Clinical Mastitis. BMC Vet. Res. 8:131.

Chapter 3.2

Between-herd prevalence of *Mycoplasma bovis* in Bulk Milk in Flanders, Belgium

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Abstract

Mycoplasma bovis is a highly infectious pathogen of cattle causing pneumonia, polyarthritis, otitis, and less frequently, subcutaneous abscesses, abortions and meningitis. Ineffective drugs treatments, culling of infected cows and loss of milk production can lead to significant economic loss on dairy farms. The early detection of cows excreting *M. bovis* bacteria to prevent mastitis outbreaks is warranted. Reports suggest that the risk of *M. bovis* mastitis is higher in larger dairy herds. The objective of this study is to estimate the herd-level prevalence of *M. bovis* in Flanders, Belgium by culturing bulk tank milk samples taken from dairy farms. Three bulk tank milk samples per dairy herd were taken over four weeks, with collection intervals of two weeks. Culturing was done after pre-incubation using modified Hayflicks media to increase the chances of recovery of bacteria. For the identification of *M. bovis*, tDNA intergenic spacer PCR was used. In three herds (1.5%) of the 200 herds sampled, *M. bovis* was isolated from one of the three consecutive bulk tank milk samples. We conclude that in Flanders in 2009 at least 1.5% of the dairy herds had one or more cows excreting *M. bovis* in the milk. The frequent monitoring of bulk tank milk to detect the presence of *M. bovis*, especially in expanding herds on farms that often purchase replacement animals, should be encouraged in order to detect the presence of *M. bovis* and to monitor the success of control procedures following an outbreak of mycoplasmal mastitis in the herd.

Introduction

Mycoplasma bovis is a highly infectious pathogen of cattle, causing pneumonia, polyarthritis, otitis, and, less frequently, subcutaneous abscesses, abortions, and meningitis (Nicholas and Ayling, 2003). In addition, it is the most important agent of outbreaks of mycoplasmal mastitis in dairy cows (Gonzalez, 2003). *Mycoplasma* spp. lacks a typical cell wall, and so are not affected by many of the commercially available antimicrobial drugs, which act by interfering with cell wall synthesis (Bushnell, 1984). Over the last decade, *M. bovis* isolates have developed an acquired resistance to a wide range of commonly used antibiotics such as macrolides and tetracyclines (Nicholas et al., 2008). Intramammary infections with *M. bovis* are difficult to treat successfully even if the antimicrobials used show good in vitro activity against the agent (Ayling et al., 2000). Unsuccessful therapy, culling of infected cows and loss of milk production can lead to significant economic loss on a dairy farm (Nicholas and Ayling, 2003). The early detection of cows excreting *M.bovis* to prevent mastitis outbreaks is warranted. Recently, several cases of clinical and subclinical mastitis caused by *Mycoplasma* spp. in Belgian dairy herds have been reported (personal communication, Milk Control Centre Flanders, Lier, Belgium). Also, the number of milk samples submitted to the central milk quality laboratory (Milk Control Centre Flanders, Lier, Belgium) for bacteriological culturing for *M. bovis* increased from zero in 2007 to 553 in 2008, (a combination of bulk milk samples and individual cow milk samples). Nearly 9% (n=48) of all samples were culture-positive (Annual Report 2008, Milk Control Centre Flanders, Lier, Belgium). No information is available on the between-herd prevalence of cows excreting *M. bovis* bacteria in Flanders, Belgium. The culture of bulk tank milk samples is a valuable procedure for screening and surveillance of mastitis-causing pathogens at the herd level, in particular for the detection of cows excreting *Streptococcus agalactiae* and *M. bovis* (Jasper, 1979; Bushnell, 1984; Gonzalez, 1986; Gonzalez, 1992; Jayarao, 2003). The objective of this study was to estimate the herd-level prevalence of *M. bovis* in Flanders, Belgium, by means of culturing of bulk tank milk samples.

Materials and Methods

The sample size required to estimate the prevalence of *Mycoplasma* spp.-infected herds accurately was calculated using Win Episcope 2.0 (Thrushfield et al., 2001). The target population was 6,287 Flemish dairy producers with an expected prevalence of *M. bovis* of 5% (± 3%), and a 95% confidence interval. Based on this calculation, the suggested adjusted sample size was 197 herds. In the 2009 year, 201 herds were selected randomly in Flanders

in proportion to the total number of dairy farmers in each of the five Provinces for this study (Table 1). Bulk milk samples were collected through routine sampling as currently performed when milk is collected as part of the legal requirements for milk quality control procedures by the Milk Control Centre Flanders, Lier, Belgium. The milk samples were immediately stored at 4°C and transported under cooled conditions (at 4°C) to the laboratory for bacteriological analysis the next day. Three bulk milk samples per herd were collected and analysed over four weeks, with collection intervals of two weeks. Culturing was performed as described by the National Mastitis Council after pre-incubation using modified Hayflicks media to increase the recovery rates of the bacteria (Hogan et al., 1999). For identification of *M. bovis*, tDNA intergenic spacer PCR was used (Stakenborg et al., 2005).

Table 1. Bulk tank milk samples required and numbers tested during a survey for *Mycoplasma bovis*, to detect a between-herd prevalence of 5% (with 95% confidence), proportionally to the total number of dairy herds per province.

Province	Recorded no. dairy herds in Flanders	No. dairy herds required	No. dairy herds included
Antwerpen	1189	37	38
Limburg	594	19	19
Oost-Vlaanderen	1752	55	56
Vlaams Brabant	407	13	13
West-Vlaanderen	2346	74	75
Total	6287	197	200[α]

[α] one farm stopped delivering milk, was only sampled once, and omitted from the results

Results & Discussion

One of the 201 selected farms stopped delivering milk during the study and was sampled only once (with a negative culture result). In the remaining 200 herds, *M. bovis* was isolated from one of the three consecutive bulk tank milk samples taken from 3 herds (1.5%). All culture-positive samples were positive at the first reading (three days after the commencement of incubation). The between-herd prevalence of *M. bovis* in bulk milk ranges between 1% and 8% in the USA (Fox et al., 2003); is 5.4% in Greece (Filiousis et al., 2007), and is nil in New Zealand (McDonald et al., 2009). False-negatives may occur, suggesting the between-herd prevalence in Flanders, Belgium may be higher than the 1.5% prevalence found in this study. Infected cows may excrete the organisms in low numbers (Gonzalez, 1986) or intermittently,

and so may not be isolated on culture (Jasper, 1979; Bushnell, 1984; Biddle, 2003). Additionally, milk from *M. bovis* infected cows in large herds will be diluted in the total herd milk. Dairy producers also often withhold abnormal milk from the milk tank (Jasper, 1979; Thomas, 1981; Gonzalez, 1992). Reports suggest that the risk of mastitis caused by *Mycoplasma* spp. is higher in large herds, presumably because cows and heifers are purchased more frequently either to maintain or expand the existing herd. (Thomas et al., 1981, Fox et al., 2003). During the last 20 years, the size of the dairy herd increased in Flanders, Belgium as illustrated by the increasing average volume of milk quota per farm (Annual Report 2010, Confederation of the Belgian Dairy Industry, Belgium). This increase in herd size is mainly driven by the acquisition of cows and heifers from other herds, a key risk factor for the introduction of *M. bovis* into a dairy herd (Jasper, 1979; Gonzalez and Wilson, 2003). As well, the average BMSCC in Flanders has increased since 1999, indicating that more attention to udder health management by farmers is required. Both of these observations indicate that the risk of *M. bovis* infection in a dairy herd has increased.

Conclusion

Our conclusion from the study is that in Flanders, Belgium in 2009, at least 1.5% of dairy herds had one or more cows excreting *M. bovis* in the milk. Frequent monitoring of bulk tank milk (especially on farms that purchase replacement animals) should be encouraged in order to screen for and detect the presence of *M. bovis* and to monitor the success of control procedures on the farm following an outbreak of mycoplasmal mastitis.

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References

Activity report, Confederation of Belgian Dairy Industry (2010), 44 – 47.

Activity report, Milk Control Centre Flanders (2008), 31.

Ayling, R.D., Baker, S.E., Peek, M.L., Simon, A.J. & Nicholas, R.A.J. (2000) Comparison of in vitro activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of Mycoplasma bovis. Veterinary Record 146, 316-318.

Biddle, M.K., Fox, L.K. & Hancock, D.D. (2003) Patterns of mycoplasmal shedding in the milk of dairy cows with intramammary mycoplasma infection. Journal of the American Veterinary Medical Association 223, 1163-1166.

Bushnell, R.B. (1984) Mycoplasma mastitis. Veterinary Clinics of North America: Large Animal Practice 6, 301-312.

Filiousis, G., Christodoulopoulos, G., Thatcher, A., Petridou, V. & Bourtzi-Chatzopoulou, E (2007) Isolation of Mycoplasma bovis from bovine clinical mastitis in Northern Greece. The Veterinary Journal 173, 215-218.

Fox, L.K., Hancock, D.D., Mickelson, A. & Britton A. (2003) Bulk tank milk analysis: factors associated with appearance of Mycoplasma spp. in milk. Journal of Veterinary Medicine B 50, 235-240.

Gonzalez, R.N., Jasper, D.E. & Farver, T.B. (1986) Relationship between mastitis pathogen numbers in bulk tank milk and bovine udder infections in California dairy herds. Journal of the American Veterinary Medical Association 189, 442-445.

Gonzalez, R.N., Merill, R.A. & Sears, P.M. (1992) Shedding of Mycoplasma bovis from the udder of naturally infected cows and its importance for the diagnosis of bovine intramammary infections. Journal of Dairy Science 75(Suppl 1), 259.

Gonzalez, R.N. & Wilson, D.J. (2002) Realistic milk culture programs for herd expansion. Proceedings of the National Mastitis Council, Orlando FL. Madison (WI): National Mastitis Council 2002, 118-124.

Gonzalez, R.N. & Wilson, D.J. (2003) Mycoplasmal mastitis in dairy herds. The Veterinary Clinics Food Animal Practice 19, 199-221.

Hoblet, K. & Iqbal, M. (1996) Mycoplasma mastitis: survey of Ohio herds. Proceedings of the 35th Annual Meeting of the National Mastitis Council, Nashville TN. Madison (WI): National Mastitis Council 1996, 77-81.

Hogan, J.S, Gonzalez, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W. & Smith, K.L. (1999) Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Inc., Madison, WI, 151-188.

Jasper, D.E., Dellinger, J.D., Rollins, M.H. & Hakanson, H.D. (1979) Prevalence of mycoplasmal bovine mastitis in California. American Journal of Veterinary Research 40, 1043-1047.

Jayarao, B.M. & Wolfgang, D.R. (2003) Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health. The Veterinary Clinics Food Animal Practice 19, 75-92.

McDonald, W.L., Rawdon, R.G., Fitzmaurice, J., Bolotovski, I., Voges, H., Humphrey, S., Fernando, K., Canagasebey, Y., Thornton, R.N. & McIntyre, L. (2009) Survey of bulk tank milk in New Zealand for Mycoplasma bovis, using species-specific nested PCR and culture. New Zealand Journal 57, 44-49.

Nicholas, R.A. & Ayling, R.D. (2003) Mycoplasma bovis: disease, diagnosis, and control. Research in Veterinary Science 74, 105-12.

Nicholas, R.A., Ayling, R.D. & McAuliffe, L. (2008). Diseases caused by Mycoplasma bovis. Mycoplasma Diseases of Ruminants. CABI, Wallingford, Oxfordshire UK, 133-154.

Stakenborg, T., Vicca, J., Verhelst, R., Butaye, P., Maes, D., Naessens, A., Claeys, G., De Ganck, C., Haesebrouck, F. & Vaneechoutte, M. (2005) Evaluation of tRNA gene PCR for identification of Mollicutes. Journal of Clinical Microbiology 43, 4558-4566.

Thomas, C.B., Willeberg, P. & Jasper, D.E. (1981) Case-control study of bovine mycoplasmal mastitis in California. American Journal of Veterinary Research 42, 511-515.

Thrusfield, M., Ortega, C., De Blas, I., Noordhuizen, J.P. & Frankena, K. (2001) Win Episcope 2.0: improved epidemiological software for veterinary medicine. Veterinary Record 148, 567–572.

Chapter 4

Milk Quality on Flemish Dairy Farms

Chapter 4.1

Manageable Risk Factors associated with Bacterial and Coliform Counts in Unpasteurized Bulk Milk in Flemish Dairy Herds

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Abstract

Associations between herd management practices and both bacterial counts (BC) and coliform counts (CC) from 254 and 242 dairy herds in Flanders (Belgium), respectively, were studied. Data were analyzed using multivariable, multilevel linear regression analysis, allowing variance components' analyses. Both BC and CC fluctuated throughout the year, although the milk quality parameters followed an opposite pattern. Bacterial count values decreased with each increase of the cleaning frequency of the cubicles (once a week, once a day, twice a day, more than twice a day) between January and March. Herds with a conventional milking parlor had substantially lower BC than herds where the cows were milked using an AMS. Lower BC were observed when the milking parlor was equipped with an automatic cluster removal system, when premilking teat desinfection was applied, when the dry cows were supplemented with a mix of minerals and vitamins, and when the teats were prepared either first wet and dried or via an AMS. Milking cows with a high-pipeline milking parlor set-up or with an AMS was associated with substantially higher CC values. Herds where prepartum heifers were often treated with antimicrobials before calving had a lower CC than farms where heifers were either not or only rarely treated. Most variation in BC and CC resided at the herd level rather than at the observation level, indicating that management is important in the control of both BC and CC. Still, only a small proportion of the total variance was explained by factors capturing information related to the milking, herd health, and dry cow management, which suggests that the bacteriological milk quality and in particular CC is primarily driven by other factors than the ones included in this study.

Introduction

High bacterial levels in milk, whether originating from the cow or the environment, substantially affect the quality, safety, and consumer acceptance of milk and dairy derived-products. Some bacteria found in unpasteurized milk such as *Staphylococcus aureus*, *Escherichia coli, Listeria monocytogenes, Campylobacter jejuni, Mycobacterium tuberculosis* and *Salmonella* spp. pose a potential risk for human health (Gilmour and Rowe, 1990; Murphy and Boor, 2000). Bacteria that are not known to be pathogenic can cause flavor-changes, rancidity and thus reduced shelf life (Boor, 2001; Barbano et al., 2006). Bacterial quality of milk can be determined using a number of parameters including BC, preliminary incubation counts, laboratory pasteurization counts, and CC (Murphy, 1997). Among these, BC is the most commonly used one in regulatory programs (Murphy and Boor, 2000) and estimate the number of colony forming units (cfu) or individual bacteria counts (IBC) present in unpasteurized bulk tank milk.

In Flanders (Belgium), the official mandatory milk quality regulations follow European legislation and require a geometric mean BC over the last two months (based on two recordings per month) < 100 x 10^3 IBC per mL milk. In contrast, testing of CC is non-obligatory for milk quality in Flanders yet implemented as part of an incentive program. Similar to other countries such as Ireland (Berry et al., 2006) and different regions in the US (Jayarao et al., 2004), the majority of milk processors in Flanders pays incentives of up to 0.75 € per 100 L of milk to farmers that meet higher quality requirements, including geometric mean BC < 50 x 10^3 IBC/mL and geometric mean CC < 50 cfu/mL over the last two months (4 recordings) in combination with a geometric mean SCC < 350 x 10^3 cells/mL, in the absence of antibiotic residues in any milk delivery; all to ensure the image of milk as a high quality and safe product. Although bulk tank BC and CC in Flanders decreased by 19.2% between 2005 and 2008, an increase of almost 10% was observed between 2008 and 2009 (Milk Control Centre, Lier, Belgium), warranting the need to understand the reasons behind this tendency.

Most studies have identified factors holding information on milking and equipment hygiene, sanitizing procedures (Elmoslemany et al., 2009a; Elmoslemany et al., 2009b; Pantoja et al., 2011) and milk storage conditions (Murphy and Boor, 2000) explaining variability in BC and CC. Apart from two studies, of which one was conducted in Chile (van Schaik et al., 2005) and the other one in Canada (Elmoslemany et al., 2010), factors related to either herd health management, transition and feeding management or housing, that are known to affect udder health, have not been studied as potential risk factors. Still, mastitis-causing streptococci such as *Streptococcus uberis* and *Streptococcus agalactiae* can be important contributors to bacterial levels of the unpasteurized bulk tank milk (Zadoks et al., 2004). Given this

information, one could anticipate that the latter management practices are also relevant for milk quality and BC in particular, and could explain the increase in BC and CC in Flanders, coincident with the increase in the average bulk milk SCC during the same period.

The main objective of this study was to evaluate to what extent differences in management practices different from those related to milking and equipment hygiene are associated with BC and CC in unpasteurized bulk milk on Flemish dairy herds. A secondary objective was to assess whether the variation in BC and CC resided mostly at the herd or at the observation level.

Materials and Methods

Herd Demographic Data

In 2009, Flanders had 6,971 dairy herds with an average herd size and milk production of 40.9 cows and 8,059 milk kg/cow per year. The average milk quota size was 310,708 kg. Herds included in this study were on average larger (65.8 cows/herd) in both size and milk production (8,503 kg milk/cow per year). The average of the geometric mean of BC and CC in Flanders were 11.3 x 10^3 IBC/mL and 10 cfu/mL, respectively. In 2009, 97% of the herds met the requirements for BC according to the European legislation (geometric mean BC < 100 x 10^3 IBC/mL) whereas approximately 85% of the herds met the specific requirements for higher quality milk for CC (geometric mean CC < 50 cfu/mL). The average of the geometric mean bulk milk SCC was 230,000 cells/mL. The milk quality of the herds included in this study was comparable.

Herd Selection and Data Collection

A written web-based questionnaire was conducted between January 2010 and July 2010. The questionnaire was pretested and fine-tuned in close cooperation with 4 dairy farmers prior to the start of the study.

In total, 254 farmers completed the online questionnaire that consisted of 39 questions concerning general management (n = 8), herd health management (n = 5), milking management (n = 11), calving (n = 3) and dry cow management (n = 6), and nutrition (n = 6), in place on farm during 2009 (Table 1).

Table 1. Overview of all herd management practices collected via a web-based questionnaire on 254 dairy herds in Belgium.

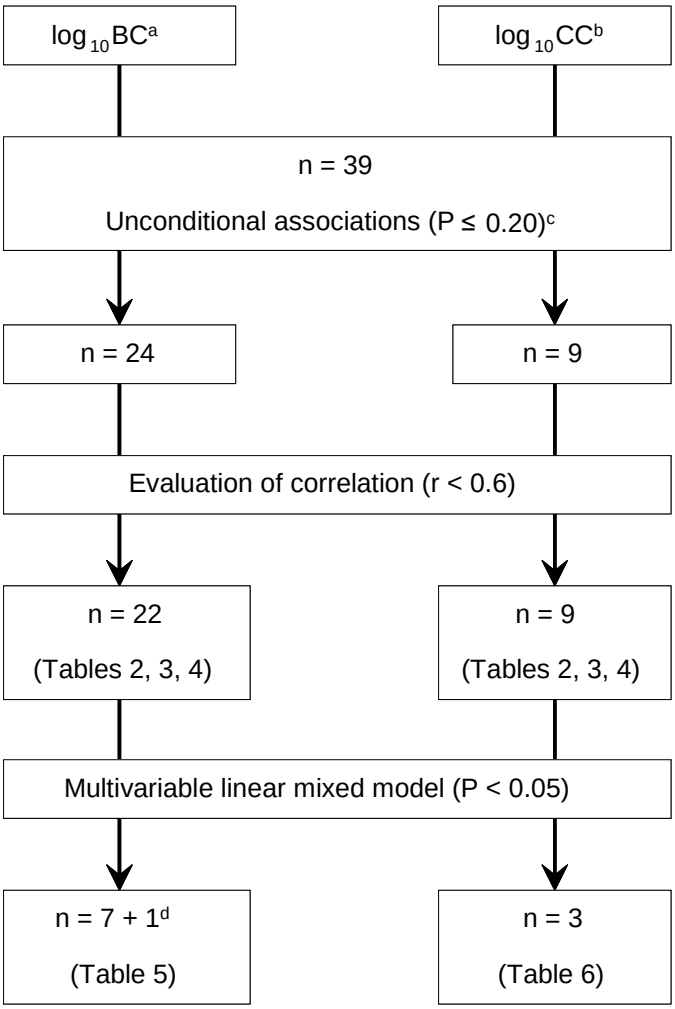
General management	
	Type of livestock farming, expected time the farm will still exist, herd size, number of lactating cows, milk quota size, duration of access to pasture during summer, barn type, cleaning frequency of the housing
Herd health management	
	Registration of animal diseases, herd health monitoring by veterinarian, monthly incidence of clinical mastitis, antimicrobial treatment during lactation of subclinical mastitis, treatment of prepartum heifers with antimicrobials
Milking management	
	Milking machine type, milking parlor type, cows kept in headlock after milking, use of automatic cluster removal, providing a prep-lag time of 60 s, teat preparation method, application of premilking teat disinfection, application of postmilking teat disinfection, machine unit liner, rinsing of machine unit liners, replacement of machine unit liners
Calving management	
	Calving on pasture, presence of calving pen, use of calving pen for sick cows
Nutrition management	
	Concentrate provided during milking, concentrate provided on top of forage, concentrate provided via TMR, concentrate provided via automatic feeder, forage provided, type of forage feeding system
Dry cow management	
	Drying off procedure, use of long-acting antimicrobials, adapted diet provided, mineral/vitamin mix provided, use of external teat sealer, use of internal teat sealer

From all farms that completed the online questionnaire, the bulk tank milk BC and CC records measured at 2-weekly intervals from January 2009 to December 2010 were retrieved from the Milk Control Center Flanders that executes the (regulatory) farm screening program in Flanders. Bacterial counts and CC were examined on unpasteurized bulk milk samples collected in 30-mL sterile screw-cap tubes by trained milk haulers. The samples were kept cooled (± 4°C) until arrival at the laboratory.

Total Bacterial and Coliform Counts

All microbiological analyses were performed within 24h after pick-up at the farm. The milk samples were vortexed prior to the start of the analyses. For BC, undiluted milk samples were used and automatically analyzed by means of a BactoScanTM FC (Foss Electric, Hillerød, Denmark). Bacterial counts were expressed as the number of IBC/ml of milk. For CC, milk

samples were first diluted using trypton salt broth at 1:10. One mL of diluted milk was plated on 3M™ Petrifilm CC plates and incubated at 30°C for 24h. Colony-forming units were counted electronically using an automated colony counter (protoCOL, Synbiosis, Cambridge, UK). Coliform counts were expressed as the number of cfu/mL of milk. Per month, a geometric mean BC and CC was calculated based on the last 4 recordings (last 2 months). A log10-transformation of the geometric mean BC and CC was performed to normalize the data before statistical analyses.



[a]Bacterial count, [b]Coliform count, [c]All univariable models were corrected for month of observation to take into account the longitudinal nature of the data, [d]Interaction between month of observation and one other variable.

Figure 1. Flow chart of variable reduction through different steps in the statistical analysis.

Table 2. Unconditional associations between general and herd health management factors and log_{10}BC and log_{10}CC on 254 and 242 Flemish dairy farms, respectively ($P \leq 0.20$).

	Bacterial count (IBC x 10^3/mL)				Coliform count (cfu/mL)		
Independent variables	Herds, N (%)		LSM[a]/β[b]	*P*-value	Herds, N (%)	LSM [a]	*P*-value
General management							
Number of lactating cows	254[b]		-0.000691[b]	0.02			NS
Expected time the farm will still exist				0.01			NS
< 5 years	30	(11.8)	8.4				
5 - 10 years	18	(7.1)	9.0				
≥ 10 years	204	(80.3)	7.2				
No idea	2	(0.8)	5.6				
Cows on pasture during summer				0.23			NS
No	30	(11.8)	6.9				
< 4h	24	(9.4)	6.8				
4h -12 h	136	(53.5)	7.7				
> 12 h	64	(25.2)	7.5				
Barn type				< 0.001			NS
Free stall with slatted floor	202	(79.5)	7.2				
Free stall with non-slatted floor	12	(4.7)	7.7				
Straw box with slatted or non-slatted floor[c]	24	(9.4)	8.1				
Tie stall with slatted or non-slatted floor	16	(6.3)	10.2				

Table 2. (continued).

Independent variables	Bacterial count (IBC x 10^3/mL)				Coliform count (cfu/mL)			
	Herds, N (%)		LSM[a]/β[b]	*P*-value	Herds, N (%)		LSM [a]	*P*-value
General management								
Cleaning frequency of cubicles				0.01				NS
1 time per week	9	(3.5)	10.8					
1 time per day	13	(5.1)	8.6					
2 times per day	174	(68.5)	7.5					
> 2 times per day	58	(22.8)	6.7					
Herd health management								
Herd health monitoring performed by veterinarian				0.02				0.10
No	108	(42.5)	7.9		104	(43.0)	8.5	
Yes	146	(57.5)	7.1		138	(57.0)	7.3	
Treatment of prepartum heifers with antimicrobials				NS				0.06
Never / rarely / sometimes					233	(96.3)	7.9	
Usually / always					9	(3.7)	4.8	
Monthly incidence of clinical mastitis in 2009				0.01				0.10
≤ 3 %	122	(48.0)	6.9		116	(47.9)	7.1	
> 3 %	132	(52.0)	7.9		126	(52.1)	8.4	

[a]Back-transformed least square means of $\log_{10}$ bacterial count (x10^3 IBC/mL) and $\log_{10}$ coliform count (CC) (cfu/mL), respectively, corrected for month of observation, [b]Regression coefficient of the model, [c]On 5 farms the barn consisted of a strawbox and some cubicles.

Table 3. Unconditional associations between milking routine and log_{10}BC and log_{10}CC on 254 and 242 Flemish dairy farms.

	Bacterial count (IBC × 10^3/mL)				Coliform count (cfu/mL)			
Independent variables	Herds, N (%)		LSM [a]	*P*-value	Herds, N (%)		LSM [a]	*P*-value
Milking management								
Milking machine type				<0.001				0.10
Low line	189	(74.4)	7.2		179	(73.9)	6.3	
High line	24	(9.4)	10.0		22	(9.1)	9.3	
With glass jars	28	(11.0)	6.9		28	(11.6)	5.6	
Automatic milking system	13	(5.1)	7.7		13	(5.4)	16.1	
Milking parlor type				<0.001				0.01
Fishbone	145	(57.3)	6.9		139	(57.6)	7.3	
Tandem	53	(20.9)	8.1		51	(21.2)	7.5	
Rotary	19	(7.5)	6.8		17	(7.1)	6.6	
Side-by-side	6	(2.4)	7.2		5	(2.1)	10.7	
Automatic milking system	13	(5.2)	8.6		13	(5.4)	17.0	
Tie stall	17	(6.7)	10.4		16	(6.6)	7.9	
Cows kept in headlocks after milking				0.17				NS
No, although possible	150	(59.1)	7.5					
Yes, but not all animals	32	(12.6)	8.1					
Yes	72	(28.3)	7.0					
Use of automatic cluster removal				<0.001				0.06
No	36	(14.2)	10.7		35	(14.5)	9.7	
Yes	218	(85.8)	7.0		207	(85.5)	7.5	

Table 3. (continued).

	Bacterial count (IBC × 10^3/mL)				Coliform count (cfu/mL)			
Independent variables	Herds, N (%)		LSM [a]	*P*-value	Herds, N (%)		LSM [a]	*P*-value
Milking management								
Method of teat preparation				0.01				NS
Wet and dry after /automatic	42	(16.5)	7.0					
Dry with paper towel	155	(61.0)	7.9					
Disinfection towels	10	(3.9)	6.2					
Dry with cloths	47	(18.5)	7.8					
Providing a prep-lag time of 60 s				<0.001				0.03
Never / rarely / sometimes	94	(37.0)	8.2		88	(36.3)	8.2	
Not applicable (rotary milking parlor)	20	(7.9)	7.5		19	(7.9)	12.2	
Always / usually / sometimes	140	(55.1)	7.0		135	(55.8)	7.1	
Application of premilking teat disinfection				0.01				NS
Never / rarely / sometimes	229	(90.2)	7.6					
Always / usually	25	(9.8)	6.0					NS
Application of postmilking teat disinfection				0.13				
Never / rarely / sometimes	27	(10.6)	8.2					
Always / usually	227	(89.4)	7.3					
Type of milking machine unit liner				0.06				NS
Rubber	229	(90.2)	7.5					
Silicone	25	(9.8)	6.5					

[a]Back-transformed least square means of $\log_{10}$ bacterial count (BC) (x10^3 IBC/mL) and $\log_{10}$ coliform count (CC) (cfu/mL), respectively, corrected for month of observation.

Table 4. Unconditional associations between nutrition, calving and dry cow management and log_{10}BC and log_{10}CC on 254 and 242 Flemish dairy farms, respectively ($P \leq 0.20$).

	Bacterial count (IBC × 10^3/mL)				Coliform count (cfu/mL)			
Independent variables	Herds, N (%)		LSM [a]	*P*-value	Herds, N (%)		LSM [a]	*P*-value
Nutrition management								
Type of forage feeding system				<0.001				NS
Layers	56	(22.0)	7.4					
Partly mixed	56	(22.0)	7.4					
Total mixed ration	131	(51.6)	7.1					
Completely separated	11	(4.3)	12.2					
Concentrate provided on top of forage				<0.001				NS
No	222	(87.4)	7.1					
Yes	32	(12.6)	9.9					
Concentrate provided via automatic feeder				<0.001				NS
No	81	(31.9)	8.4					
Yes	173	(68.1)	7.0					
Dry cow management								
Drying off procedure				0.11				NS
Intermittent milking	20	(7.9)	7.9					
Abrupt	234	(92.1)	7.3					
Adapted diet provided				0.01				0.08
No	40	(15.7)	8.8		40	(16.3)	9.3	
Yes	214	(84.3)	7.2		202	(83.7)	7.5	

Table 4. (continued).

	Bacterial count (IBC × 10^3/mL)				Coliform count (cfu/mL)			
Independent variables	Herds, N (%)		LSM [a]	*P*-value	Herds, N (%)		LSM [a]	*P*-value
Dry cow management								
Mineral/vitamin mix provided				<0.001				NS
No	67	(26.4)	9.1					
Yes	187	(73.6)	6.9					
Calving management								
Presence of calving pen				0.01				NS
No (cows calve in tie stall / cubicles)	45	(17.7)	8.5					
Yes	209	(82.3)	7.2					
Calving pen used for sick cows				0.01				NS
No	76	(29.9)	7.4					
No, as there is no calving pen	39	(15.4)	8.9					
Yes	139	(54.7)	7.1					

[a]Back-transformed least square means of $\log_{10}$ bacterial count (BC) (x10^3 IBC/mL) and $\log_{10}$ coliform count (CC) (cfu/mL), respectively, corrected for month of observation.

Statistical Analysis

All data were entered in an electronic spreadsheet program (Excel 2010, Microsoft Corporation) and were checked for unlikely values. Monthly geometric means for BC (n = 3046) and CC (n = 2895) were available for 254 and 242 of the farms, respectively.

The regression-model building process to identify management practices associated with BC and CC involved several steps as described previously (De Vliegher et al., 2004) and is presented in a flow chart (Figure 1). Linear mixed regression models with BC and CC as dependent variables were fit using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA). In all models, herd was included as random effect to model the repeated measurements within herds using a first-order autoregressive correlation structure.

Initially, unconditional associations were tested between the continuous dependent variables at the herd level, $\log^{10}$BC and $\log^{10}$CC, respectively, and all management practices (n = 39, Table 1) and month of observation as fixed effect, the latter to take into account the longitudinal nature of the data. Statistical significance at this step was assessed at $P < 0.20$ (Maldonado and Greenland, 1993). Some of the categorical variables were recoded based on biological reasons because of low frequencies in one or more categories in this step. Second, Pearson and Spearman's rank correlation coefficients were calculated among the significant independent variables to avoid multicollinearity in the next steps. If two management practices had a correlation coefficient > 0.6, only one was withheld for further analysis. In the third step, multivariable models were built with the remaining management variables as independent variables, and with log10BC and log10CC, respectively, as dependent variables (Tables 2, 3 and 4). Non-significant variables were removed using backwards elimination at $P \leq 0.05$. In both models, all first order interactions among month of observation and the remaining variables in the multivariable model were tested and removed when non-significant (Wald's tests, $P > 0.05$). The adequacy of the final model was tested by examining normal probability plots of residuals and plots of residuals versus predicted values to check whether the assumptions of normality and homogeneity of variance had been fulfilled. No patterns indicating heteroscedasticity were revealed.

To evaluate the proportion of variance occurring at the different levels of the data hierarchy, two-level null models (intercept and month of observation as fixed effect only, to take into account the longitudinal nature of the data), with herd as random effect were fit for both BC and CC.

Results

Descriptive Statistics

The average herd size of the 254 herds included in the study was 65.8 [interquartile range (IQR) from 45 to 80] lactating cows per herd with an average milk quota size of 551,937 kg milk (IQR from 375,750 to 676,500) per year. The average of the geometric mean BC was 9.1 x 10^3 IBC/mL (IQR from 5 x 10^3 to 11 x 10^3 cfu/mL) and the average of the geometric mean CC was 21.3 cfu/mL (IQR from 2 to 16 cfu/mL). Approximately 94% of the herds met the specific requirements for higher quality milk in Belgium for BC (geometric mean BC < 50 x 10^3 IBC/mL), whereas 98.4 % of the herds met the requirements according to the European legislation (geometric mean BC < 100 x 10^3 IBC/mL) for the whole year. However, 38.5 % of the herds exceeded at least once in the studied years the specific requirements for higher quality milk for CC (geometric mean CC < 50 cfu/mL). Average BMSCC was 223,621 cells/mL with an IQR from 173,000 to 271,000 cells/mL. Similar figures were obtained using the subset of 242 farms of which monthly geometric mean CC values were available for further analyses.

Of the 254 herds, 60.2 % (n = 153) only housed dairy cows, 22.9 % (n = 58) also kept double-purpose or beef cows, and 16.9 % (n = 43) also farmed other animals such as pigs. On nearly 60 % of the herds (n = 146), some herd health aspects such as fertility, and/or udder health, and/or heifer rearing, and/or claw health were monitored by a veterinarian on a regular basis. Disease registration was systematically done on approximately half of the herds. Almost 80 % of the farms (n = 202) housed their cows in a free stall with slatted floor.

Unconditional Associations

A first reduction based on unconditional associations and highly correlated variables, revealed 22 and 9 herd management practices to be associated with $\log^{10}$IBC and $\log^{10}$CC, respectively (Tables 2, 3 and 4). A strong correlation was found between the number of cows, the number of lactating cows, and the milk quota size. The number of lactating cows was selected for further analysis.

Table 5. Final multivariable, multilevel model for log_{10}BC of 254 Flemish dairy farms.

Independent variables	β[a]	SE [b]	LSM [c]	*P*-value[e]
Intercept	1.20	0.079	…	< 0.001
Month of observation				< 0.001
January	Ref.[d]	….	10.0	
February	0.100	0.049	11.6	0.04
March	0.016	0.065	11.3	0.80
April	-0.148	0.075	10.2	0.05
May	-0.197	0.082	9.2	0.02
June	-0.280	0.087	7.6	0.01
July	-0.367	0.090	6.8	< 0.001
August	-0.274	0.093	7.3	0.01
September	-0.216	0.094	7.4	0.02
October	-0.227	0.096	7.3	0.02
November	-0.200	0.097	7.8	0.04
December	-0.105	0.064	8.9	0.29
General management				
Cleaning frequency of the housing				0.22
1 time per week	Ref.	…	10.1	…
1 time per day	-0.114	0.094	8.2	0.22
2 times per day	-0.212	0.074	8.5	0.01
> 2 times per day	-0.312	0.078	7.9	0.01
Milking management				
Milking parlor type				< 0.001
Fishbone	Ref.	…	7.6	…
Tandem	0.056	0.022	8.6	0.01
Rotary	0.003	0.034	7.6	0.92
Side-by-side	0.063	0.059	8.7	0.28
Automatic milking system	0.179	0.045	11.4	< 0.001
Tie stall	0.048	0.041	8.4	0.24
Use of automatic cluster removal				< 0.001
No	Ref.	…	10.3	…
Yes	-0.148	0.029	7.3	
Teat preparation method				0.01
Wet and dry after / automatic	Ref.	…	7.9	…
Dry with paper towel	0.082	0.028	9.6	0.01
Disinfection towels	0.037	0.052	8.7	0.47
Dry with cloths	0.025	0.033	8.4	0.43
Application of premilking teat disinfection				0.01
Never / rarely / sometimes	Ref.	…	9.3	…
Always / usually	-0.063	0.030	8.0	0.04
Dry cow management				
Mineral/vitamin mix provided				0.01
No	Ref.	…	9.2	…
Yes	-0.057	0.021	8.1	
Month of observation × Cleaning frequency of barn[f]				0.01

[a]Estimate, [b]Standard error, [c]Back-transformed least square means of log_{10} bacterial count (x10^3IBC/mL), [d]Reference, [e]*P*-value of the fixed effects, [f]See Figure 2 for more detailed information.

Table 6. Final multivariable, multilevel model for log_{10}CC of 242 Flemish dairy farms.

Independent variables	β[a]	SE [b]	LSM [c]	*P*-value
Intercept	0.694	0.039	…	< 0.001
Month				< 0.001
January	Ref.[d]	…	4.9	…
February	-0.020	0.028	4.7	0.47
March	-0.043	0.036	4.5	0.01
April	-0.007	0.042	4.9	0.24
May	0.026	0.045	5.3	0.86
June	0.073	0.047	5.9	0.11
July	0.232	0.048	8.4	< 0.001
August	0.309	0.049	10.1	< 0.001
September	0.260	0.050	9.0	< 0.001
October	0.213	0.051	8.1	< 0.001
November	0.201	0.051	7.9	< 0.001
December	0.137	0.051	6.8	0.01
Milking management				
Milking machine type				< 0.001
Low-line	Ref.	…	5.7	…
High-line	0.171	0.076	9.6	0.03
With glass jars	-0.064	0.069	4.9	0.35
Automatic milking system	0.419	0.098	13.8	< 0.001
Herd health management				
Treatment of prepartum heifers with antimicrobials				0.02
Never / rarely / sometimes	Ref.	…	8.7	…
Usually / always	-0.261	0.115	4.9	…

[a]Estimate, [b]Standard error, [c]Back-transformed least square means of log_{10} coliform count (cfu/mL) obtained from the final model, [d]Reference.

Final multivariable, multilevel Models

The final multivariable, multilevel models for log^{10}BC and log^{10}CC are presented in Tables 5 and 6, respectively. Although the correlation coefficient between type of milking machine and type of milking parlor did not exceed the cut-off value of 0.6 (R = 0.27), a multivariable model for log^{10}BC in which both variables were included did not reach convergence as all tie stall milking parlors were obviously equipped with a high-pipeline set-up (Table 7). Therefore, it was decided to exclude the variable type of milking machine from the multivariable model for log^{10}BC.

Table 7. Cross tabulation of the two independent variables milking machine type and milking parlor type.

	Milking machine type				
Variable	Low-line	High-line	With glass jar	Automatic milking system	Total
Milking parlor type					
Fishbone	116	4	25	0	145
Tandem	48	3	2	0	53
Rotary	18	0	1	0	19
Side-by-side	6	0	0	0	6
Automatic milking system	0	0	0	13	13
Tie stall	0	17	0	0	17
Total	188	24	28	13	253

Factors associated with Bacterial Counts. Individual bacteria count varied throughout the year with the highest values being observed from January to March and the lowest values from June until November ($P < 0.001$). Individual bacteria count values decreased with each increase of the cleaning frequency of the cubicles (once a week, once a day, twice a day, more than twice a day) between January and March (interaction between Month of observation × Cleaning frequency of cubicles, $P < 0.05$) (Figure 2). When cows were milked using an AMS, BC were higher than on other herds using different systems ($P < 0.001$). A lower BC was observed when the milking parlor was equipped with an automatic cluster removal system as opposed to when the milking clusters were removed manually ($P < 0.001$). Lower BC was observed on herds where the teats were prepared either first wet and then dried or via an AMS ($P < 0.05$). Premilking teat disinfection via dipping or spraying as well as supplementation of dry cows with minerals were associated with lower BC as well ($P < 0.05$).

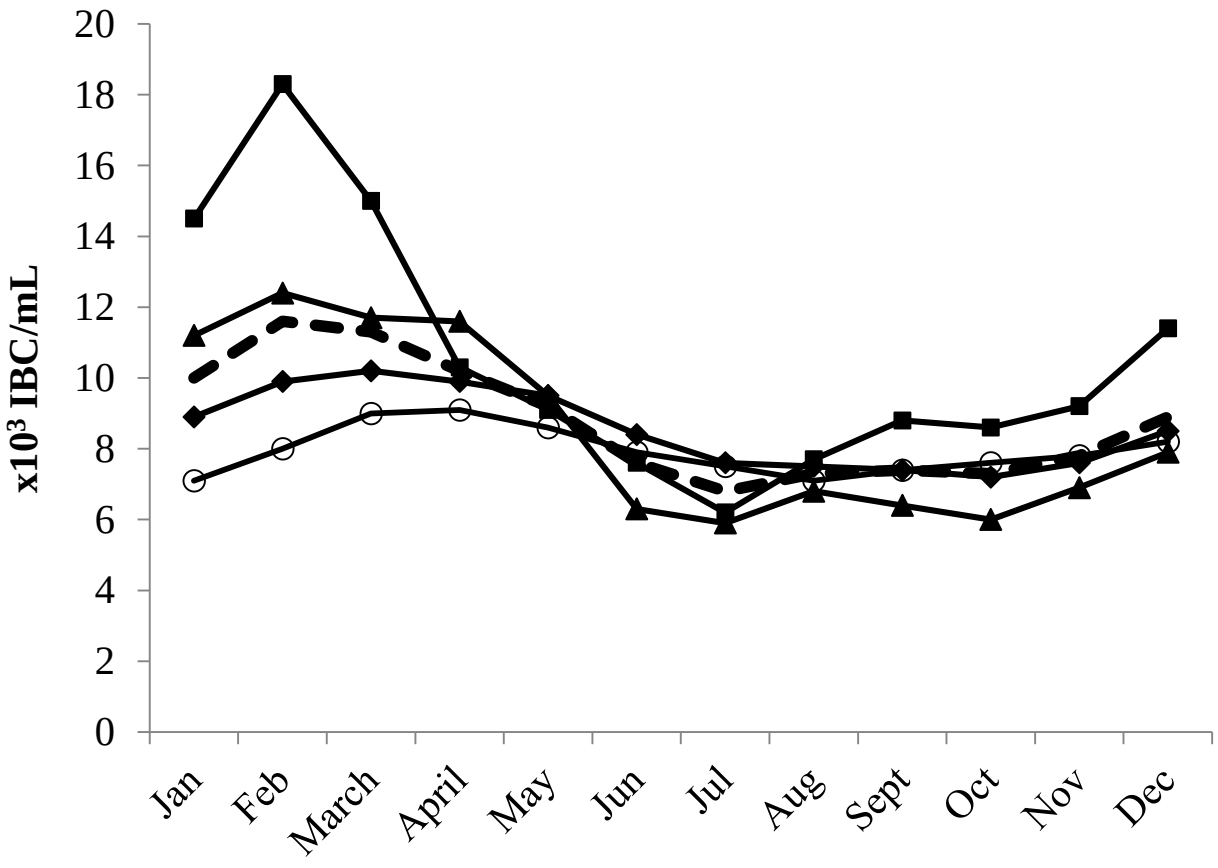


Figure 2. Back-transformed least square means of $\log_{10}$ bacterial count (BC) (x 10^3 IBC/mL) throughout the year (**- - - -**) for herds were the cubicles were cleaned once per week (■), once per day (▲), two times per day (**♦**), or more than two times per day (○).

Factors associated with Coliform Counts. Coliform counts varied throughout the year ($P < 0.001$) (Figure 3). Herds with a high-line milking parlor set-up or AMS had substantially higher CC compared to herds using a low-line milking parlor set-up or reservoir ($P < 0.001$). Farms that treated prepartum heifers with antimicrobials before calving had lower CC than farms where heifers were either not or only rarely treated before calving ($P < 0.05$).

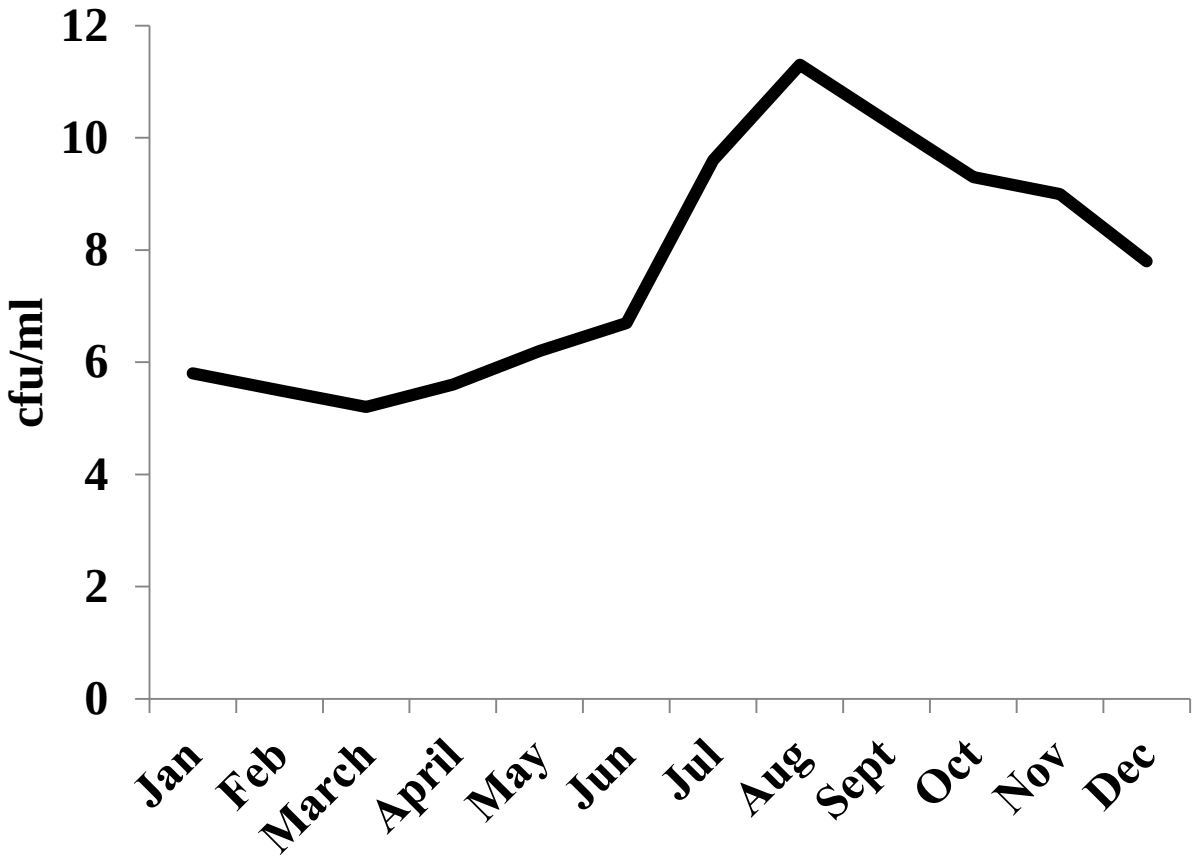


Figure 3. Back-transformed least square means of $\log_{10}$ coliform count (CC) (cfu/mL) throughout the year.

Variance Components

When fitting null-models 93.6 % of the variation in BC occurred at the herd level and 6.4 % at the observation level, whereas for CC 68.1% and 31.9% of the variation occurred at the herd and observation level, respectively (Table 8). These proportions only changed slightly in the final multivariable models. Of the total variance in BC and CC, only 4.6% and 2.1% of the variation, respectively, was explained by the fixed effects in the final models.

Table 8. Variance components at each level of the null and final multivariable models for log^{10}BC and log^{10}CC, respectively.

Data Hierarchy	Null model[a]			Final model[b]		
	Estimate	SE [c]	% [d]	Estimate	SE [c]	% [d]
Bacterial count (BC)						
Herd level	0.798	0.010	93.6	0.767	0.011	94.3
Observation level	0.054	0.003	6.4	0.046	0.002	5.7
Total variance	0.852	…	100	0.813	…	100
Coliform count (CC)						
Herd level	0.719	0.012	68.1	0.709	0.013	68.6
Observation level	0.337	0.014	31.9	0.325	0.014	31.4
Total variance	1.056	…	100.0	1.034	…	100.0

[a]Model containing only "month of observation" as fixed effect to take the longitudinal nature of the dataset in to account, [b]Model containing all fixed effects as presented in Tables 5 and 6 , [c]Standard error of the variance estimate of the parameter, [d]Variance proportion explained at the level of the data hierarchy.

Discussion

A number of studies have identified risk factors associated with milk quality parameters such as BC and CC (Elmoslemany et al., 2009a; Elmoslemany et al. 2009b; Pantoja et al., 2011). Apart from two studies of which one conducted in the 10th region of Chile (van Schaik et al., 2005) and another on Prince Edward Island in Canada (Elmoslemany et al., 2010), most studies focused on factors associated with milking equipment and cow hygiene rather than on factors related to herd health in general. In this study, a number of manageable risk factors associated with BC and CC that can be readily implemented by the Flemish dairy farmer in order to control BC and CC at acceptable levels were identified.

Web-based questionnaires are commonly used tools as they allow for relatively easy and cheap data collection. This approach has allowed us to include data of farms that were distributed over the 5 different Flemish provinces. The need to have internet access, however, might have selected the input of more contemporary farmers that are also better managers, potentially diminishing the external validity of the data (Dohoo et al., 2009). However, the high variation in BMSCC between the respondents suggests a wide diversity of management styles (Barkema et al., 1998). Also, both the average BMSCC and BC of the selected herds approached the average BMSCC and BC in 2009 for the whole of Flanders being 230,000 cells/mL and 11,3 x 10^3 IBC/mL, respectively (Annual Report 2009, Milk Control Center, Lier, Belgium). Altogether, the results are believed to reflect the BC and CC status of typical dairy

herds in Flanders and are likely valid for the Flemish dairy situation and herds of other regions and countries with similar management conditions.

The average BC in our study was slightly lower than the 11.4 x 10^3 IBC per mL found in the New York State (van Schaik et al., 2002). The average CC, on the other hand, was almost half of the average CC of 44 cfu/mL across a number of studies (e.g. Elmoslemany et al., 2009b; Pantoja et al., 2011), suggesting better conditions on the Flemish dairy farms compared to the farms included in the other studies (Jayarao and Wolfgang, 2003). As was expected the average CC and BC strongly varied among herds which is comparable with other studies (Elmoslemany et al., 2009a; Elmoslemany et al., 2009b; Pantoja et al., 2011; Mallet et al., 2012).

Several variables reflecting milking, herd health and dry cow management were significantly associated with either BC or CC, but explained only a small proportion of their total variance. The latter suggests that the bacteriological milk quality and then in particular the CC is primarily driven by other management practices than those studied, such as factors related to milking equipment and milk storage, although unknown factors should be suspected as well. Obviously, the fact that a number of associations were statistically significant in the analyses not necessarily indicates that a causal relation exists (Dohoo et al., 1997). Rather, some associations need confirmation, whereas others such as the association between prepartum antibiotic treatment in heifers and CC as well as the negative association between feeding diets supplemented with minerals to the dry cows and BC generate new hypotheses on controlling BC and CC that should be tested.

Bacterial counts and CC changed over time. Bacterial counts were highest during the winter and spring months opposed whereas CC followed the opposite pattern. In other studies conducted in the Balearic Islands, Chile, and Prince Edward Islands, both BC and CC were highest in summer (Soler et al., 1995; van Schaik et al., 2002; Elmoslemany et al., 2010). The discrepancy between our results and those reported by others can yet not be fully explained, but might be related to the temperate maritime climate in Belgium including ample rainfall all year around. The fact that cows on Flemish dairy farms are in confined housing from October up to May might expose the udder and teats under the rather mild and humid weather conditions to high bacterial levels, increasing the risk for contamination of the udder and teats in the winter and spring months. The opposite pattern of CC compared to BC indicates that both are partly determined by other factors. Coliforms only start to grow and multiply when the ambient temperature exceeds 10°C (Naemura and Seidler, 1978). Accumulation of soil from the teats and udders somewhere in the milking equipment will therefore have a larger impact on CC in summer than in winter time.

The dose-response relationship of frequency of cleaning of the housing on BC between January and March corresponded well with the data published by Kelly et al. (2009). Frequency of bedding change has been associated with the cleanliness of udder and teats and thus the exposure of teats to bacteria before (Ward et al., 2002). Also, dirty teats increase the risk of elevated BC (Elmoslemany et al., 2009a). Keeping in mind the confinement-housing and the mild and wet Belgian weather conditions in winter time, farmers should, in particular during those months, put more effort in keeping the housing of the lactating cows clean.

The higher BC and CC levels on farms equipped with an AMS are complementary with the increasing bulk milk BC levels observed on farms switching from conventional to automatic milking (Rasmussen et al, 2002; de Koning et al., 2003). The increase in BC shortly after introduction of the AMS has been suggested to partly originate from contamination of milk from the teat surfaces and partly from the inappropriate cleaning of the milking equipment and cooling of the milk (Rasmussen et al., 2002). The CC was also affected by the type of milking machine. Higher CCs were observed in milking parlors with a high-pipeline set-up compared with those with a low-pipeline set-up or reservoir (Reineman et al., 2003). A common denominator among the studies that have yet been performed is the overriding importance of proper cleaning and sanitation of the milking system in producing high quality milk (Elmoslemany et al., 2009a; Pantoja et al., 2011). The cleaning and sanitation of the pipelines in stalls with a high-pipeline set-up are more often lacking than in other milking systems likely due to the higher risk for accumulation of milk fat and drying of milk in the high-pipeline set-up and in particular in the cool line at the high end of the system (Reineman et al., 2003).

Individual bacteria count was affected by premilking teat cleaning practices which was not that unexpected as Elmoslemany et al. (2009a) reported that the risk of having high BC increased by 5.3 for each unit increase in the teat-end cleanliness before the milking unit was attached. In our study, a wet teat preparation followed by dry wiping was associated to lower BC than dry wiping with a paper towel alone, corresponding well with the results of Kelly et al. (2009). Still, in almost 25% of the cases the wet teat preparation consisted of premilking teat disinfection which was associated with lower BC as well which is obviously positive. The latter finding corresponds well to the numerous studies that showed a strong reducing effect of premilking teat disinfection on the teat microbial load (e.g. Galton et al., 1986; Magnusson et al., 2006; Gibson et al., 2008).

A negative relationship was found between the presence of automatic removal and BC which is in accordance with the results reported by Kelly et al. (2009). This finding might be related to the increased risk for teat end callosity and hyperkeratosis in case of manual cluster removal. Teats with a highly calloused teat end have an increased load of environmental

pathogens (Paduch et al., 2012). Also, teats with a distinct callosity ring are most likely less effectively cleaned during premilking teat preparation, leaving more bacteria on the teat end when the milking cluster is attached (Hovinen et al., 2005). Although little peer-reviewed publications are available on the risk factors associated with teat-end callosity, it is reasonable to accept that manual cluster removal might more often lead to overmilking compared to automatic cluster removal, thereby compromising the teat-end quality (Rasmussen et al., 1991; Gleeson et al., 2003).

Conclusions

Multivariable, multilevel regression analysis revealed a number of management practices associated with either BC or CC. Increasing the cleaning frequency of the housing during winter time under the Belgian weather conditions and implementing premilking teat disinfection by either dipping or spraying the teats before attaching the milking unit will likely result in lower BC values. The variation in both BC and CC seems to be mainly determined by differences in management between the herds as most variation in both parameters resided at the herd rather than at the observation level. As only a small proportion of the total variance was explained by the management practices included in our study, bacteriological milk quality seems to be primarily driven by other factors than the ones included in this study.

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References

Barbano, D. M., Y. Ma, and M. V. Santos. 2006. Influence of raw milk quality on fluid milk shelf life. J. Dairy Sci. 89 suppl.1:15-19.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, G. Benedictus, and A. Brand. 1998. Management practices associated with low, medium, and high somatic cell counts in bulk milk. J. Dairy Sci. 81:1917-1927.

Berry, D. P., B. O'Brien, E. J. O'Callaghan, K. O. Sullivan, and W. J. Meaney. 2006. Temporal trends in bulk tank somatic cell count and total bacterial count in Irish dairy herds during the past decade. J. Dairy Sci. 89:4083-4093.

Boor, K. J. 2001. Fluid dairy product quality and safety: Looking to the future. J. Dairy Sci. 84:1-11.

De Vliegher S., H. Laevens, H. W. Barkema, I. R. Dohoo, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Management practices and heifer characteristics associated with early lactation somatic cell count of Belgian dairy heifers. J. Dairy Sci. 87:937-947.

Dohoo, I. R., C. Ducrot, C. Fourichon, A. Donald, and D. Hurnik. 1997. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29:221-239.

Dohoo, I., M. Wayne, and H. Stryhn (Eds.), 2009. Questionnaire design. In: Veterinary Epidemiologic Research 2nd Edition, VER Incorporated, Charlottetown, Canada p. 58-72.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, and B. M. Jayarao. 2009a. Risk factors for bacteriological quality of bulk tank milk in Prince Edwards Island dairy herds. Part 1: Overall risk factors. J. Dairy Sci. 92:2634-2643.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, and B. M. Jayarao. 2009b. Risk factors for bacteriological quality of bulk tank milk in Prince Edwards Island dairy herds. Part 2: Bacteria count-specific risk factors. J. Dairy Sci. 92:2644-2652.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, J. J. Wichtel, H. Stryhn, and R. T. Dingwell. 2010. The association between bulk milk analysis for raw milk quality and on-farm management practices. Prev. Vet. Med. 95:32-40.

Galton, D. M., L. G. Petersson, and W. G. Merill. 1986. Effects of premilking udder preparation practices on bacterial counts in milk and on teats. J. Dairy Sci. 69:260-266.

Gibson, H., L. A. Sinclair, C. M. Brizuela, H. L. Worton, and R. G. Protheroe. 2008. Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. Lett. Appl. Microbiol. 46:295-300.

Gilmour, A. and M. T. Rowe. 1990. Micro-organism associated with milk. Pages 37-114 in Dairy Microbiology. 1st ed. R. K. Robinson, ed. Elsevier Science Publishers, London.

Gleeson, D. E., E. J. O'Callaghan, and M. Rath. 2003. The effects of genotype, milking time and teat-end vacuum pattern on the severity of teat-end hyperkeratosis. Ir. J. Agri. Food Res. 42:195-203.

Hovinen, M., A. M. Aisla, and S. Pyörälä. 2005. Visual detection of technical success and effectiveness of teat cleaning in two automatic milking systems. J. Dairy Sci. 88:3354-3362.

Jayarao, B. M., and D. R. Wolfgang. 2003. Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health. Vet. Clin. North Am.: Food Anim. Pract. 19:75-92.

Jayarao, B. M., S. R. Pillai, A. A. Sawant, D. R. Wolfgang, and N. V. Hegde. 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87:3561-3573.

Kelly P. T., K. O'Sullivan, D. P. Berry, S. J. More, W. J. Meaney, E. J. O'Callaghan, and B. O'Brien. 2009. Farm management factors associated with bulk tank total bacterial count in Irish dairy herds during 2006/07. Ir. Vet. J. 62:36-42.

de Koning, K., B. Slaghuis, and Y. van der Vorst. 2003. Robotic milking and milk quality: effects on bacterial count, somatic cell counts, freezing point and free fatty acids. Ital. J. Anim. Sci. 2:291-299.

Magnusson, M., A. Christiansson, B. Svensson, and C. Kolstrup. 2006. Effect of different premilking manual teat-cleaning methods on bacterial spores in milk. J. Dairy Sci. 89: 3866-3875.

Maldonado, G., and S. Greenland. 1993. Simulation study of confounder-selection strategies. Am. J. Epidemiol. 138:923-936.

Mallet, A., M. Gueguen, F. Kauffmann, C. Chesneau, A. Sesboue, and N. Desmasures. 2012. Quantitative and qualitative microbial analysis of raw milk reveals substantial diversity influenced by herd management practices. Int. Dairy J. 27:13-21.

Murphy, S.C. 1997. Raw milk bacteria tests: Standard plate count, preliminary incubation count, lab, pasteurization count and coliform count. What do they mean for your farm? Pages 34-41 in Natl. Mastitis Council. Reg. Mtg. Proc., Syracuse, New York. Natl. Mastitis Council, Inc., Madison, WI.

Murphy, S. C., and K. J. Boor. 2000. Trouble-shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ. Sanit. 20:606-611.

Naemura, L. G., and R. J. Seidler. 1978. Significance of low-temperature growth associated with the fecal coliform response, indole production, and pectin liquefaction in Klebsiella. Appl. Environ. Microb. 35: 392-396.

Paduch, J-H., E. Mohr, and V. Krömker. 2012. The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. Vet. Micro. 158:353-359.

Pantoja, J. C. F., D. J. Reinemann, and P. L. Ruegg. 2011. Factors associated with coliform count in unpasteurized bulk milk. J. Dairy Sci. 94: 2680-2691.

Rasmussen, M. D., D. M., Galton, and L. G. Petersson. 1991. Effects of premilking teat preparation on spores of anaerobes, bacteria, and iodine residues in milk. J. Dairy Sci. 74:2472-2478.

Rasmussen, M. D., M. Bjerring, P. Justesen, and L. Jepsen. 2002. Milk quality on Danish farms with automatic milking systems. J. Dairy Sci. 85:2869-2878.

Reinemann, D. J., G. M. V. H. Wolters, P. Billon, O. Lind, and M. D. Rasmussen. 2003. Review of practices for cleaning and sanitation of milking machines. Bull. 381. Int. Dairy Fed., Brussels, Belgium.

van Schaik, G., M. Lotem, and Y. H. Schukken. 2002. Trends in somatic cell counts, bacterial counts, and antibiotic residue violations in New York state during 1999-2000. J. Dairy Sci. 85:782-789.

van Schaik G., L. E. Green, D. Guzman, E. Esparza, and N. Tadich. 2005. Risk factors for bulk milk somatic cell counts and total bacterial counts in smallholder dairy farms in the 10th region of Chile. Prev. Vet. Med. 67:1-17.

Soler, A., C. Ponsell, M. De Paz, and M. Nunez. 1995. The microbiological quality of milk produced in the Balearic Islands. Int. Dairy J. 5:69-74.

Ward, W. R., J. W. Hughes, W. B. Faull, P. J. Cripps, J. P. Sutherland, and J.E. Sutherst. 2002. Observational study of temperature moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. Vet. Rec. 151:199-206.

Zadoks, R. N., R. N. Gonzales, K. J. Boor, and Y. H. Schukken. 2004. Mastitis-causing streptococci are important contributors to bacterial count in raw bulk tank milk. J. Food Prot. 67:2644-2650.

Chapter 4.2

Management Practices associated with achieving Milk Quality Premiums in Flemish Dairy Herds

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Abstract

Associations between herd management practices, collected through a web-based questionnaire from 242 dairy herds in Flanders (Belgium), and achieving of milk quality premiums (MQP) were studied. Monthly geometric means for bulk milk somatic cell count (BMSCC), bacterial count (BC) and coliform count (CC) were available, as well as monthly test results of freezing point, residues and filtration. Data were analyzed using binary logistic regression. Only 52.9% (n = 128) of the herds achieved their milk quality premium in all (n = 12) months in 2009. If the BMSCC threshold would have been 250 x 10^3 rather than 350 x 10^3 cells/mL in a hypothetical milk quality premium system (hMQP), only 23.5% (n = 57) would have achieved their milk quality premium every month of 2009. The main reason for not achieving the milk quality premiums in 2009 in the current system was CC, whereas this would have been BMSCC in the hMQP system. Lowering the threshold from 350 x 10^3 to 250 x 10^3 cells/mL in the latter system, would have most likely been accompanied with lower BC and CC values. The final model revealed that herds where premilking teat disinfection was applied and where dry cows received minerals were significantly more likely to achieve the MQP in 2009. On the other hand, herds with a high estimated incidence of clinical mastitis (> 3%) and equipped with an automatic milking system (AMS) were significantly less likely to achieve the MQP.

Introduction

The production of high-quality milk begins at the farm. Milk quality is important with implications for human health, milk processing and on-farm profitability (More, 2009). It involves multiple factors related to cow health and udder hygiene, hygiene of the environment in which cows are housed and milked, herd health management, and nutrition. Elevated somatic cell count (SCC) lead to decreased raw milk quality and has been associated with decreased shelf life of dairy products and lower cheese yields (Klei et al., 1998; Barbano et al., 2006). Also, high bacterial levels in milk, whether originating from the cow or through contamination from the environment, substantially affect the quality, safety, and consumer acceptance of milk and dairy derived-products (Keefe and Elmoslemany, 2007; Flores-Miyamoto et al., 2014). Poor milk hygiene also undermines the trust in milk as a healthy product and raises consumer concerns with regard to human health, bacterial contamination, and antimicrobial residues (Ruegg and Tabone, 2000; Saville et al., 2000; Jayarao and Henning, 2001; Hogan, 2005; Straley et al., 2006). Of these, SCC is the most important single indicator of milk quality, reflecting the health status of the mammary gland and the risk of non-physiological changes to milk composition (Dohoo and Leslie, 1991). It is also the key component of national and international regulation programs for milk quality (van Schaik et al., 2002).

Payment schemes are an important incentive in controlling all bulk milk quality parameters (Veerkamp et al., 1998). Premium policies motivate farmers to produce high quality milk without providing a potential disruption to the milk supply. The combination of a penalty and a premium has been shown to provide a strong incentive for improvement of milk quality (Nightingale et al., 2008) and are also effective motivators to enhance management practices on dairy farm; for example, mastitis management (Valeeva et al., 2007) improved after changes in the penalty system related to exceeded levels of SCC (Schukken et al., 1992; Nightingale et al., 2008).

In Flanders (Belgium), the official mandatory milk quality regulations follow European legislation and require a geometric mean BMSCC over the last three months (based on 4 recordings per month) < 400 x 10^3 cells/mL, a geometric mean bacterial count (BC), expressed as individual bacterial count per mL (IBC/mL) over the last two months (based on two recordings per month) < 100 x 10^3 IBC/mL milk, a geometric mean of the freezing point over the last two months (based on all deliveries) > -510 m°C, no visible impurities (filtration test done once a month) and absence of antibiotic residues in any milk delivery. Testing of the CC is non-obligatory for milk quality in Flanders yet implemented as part of an incentive program.

Similar to other countries such as Ireland (Berry et al., 2006) and different regions in the US (Jayarao et al., 2004), the majority of milk processors in Flanders pays incentives of up to €1 per 100 L (which represents approximately 3% of the milk price) of milk to farmers that meet the higher quality requirements. Reaching all monthly standards for milk quality premiums and avoiding penalties obviously has potential to increase the farmer's income but definite figures have not been published. Until now, there is also little information available on which management practices predispose a farm to the loss of premiums due to inferior milk quality.

The aim of this study was to determine to what extent differences in management practices were associated with achieving milk quality premiums each month during the year 2009 on Flemish dairy herds. As a secondary objective, we examined what management practices were associated with achieving milk quality premiums if the BMSCC threshold was 250 x 10^3 rather than 350 x 10^3 cells/mL, leaving the other thresholds unchanged.

Materials and Methods

Herd Selection and Data Collection

A web-based questionnaire was conducted between January 2010 and July 2010. The questionnaire was pre-tested and refined in close cooperation with 4 dairy farmers prior to the start of the study. An invitation to fill out the questionnaire was emailed to approximately 1000 farmers based on a list from the largest farmer's organization in Flanders and an incentive (USB-stick) for a completed questionnaire was provided.

In total, 242 farmers (4% of Flemish farmers) completed the online questionnaire that consisted of 43 questions concerning general management (n = 8), herd health management (n = 9), milking practices (n = 11), calving and dry cow management (n = 9) and nutrition (n = 6), in place on farm during 2009 (Table 1).

From all farms that completed the online questionnaire, the BMSCC, BC and CC records measured between January 2009 and January 2010, as well as the results of the filtration test, the freezing point and residue test, were retrieved from the Milk Control Centre Flanders (Lier, Belgium) that executes the regulatory farm screening program in Flanders. All analyses were examined on unpasteurized bulk milk samples collected in 30 mL sterile screw-cap tubes by trained milk haulers. The samples were kept cooled (± 4°C) until arrival at the laboratory.

Laboratory Analyses

All microbiological analyses were performed within 24h after pick-up at the farm. The milk samples were vortexed prior to the start of the analyses. Milk SCC was quantified by electronic counting using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) at the Milk Control Centre Flanders (Lier, Belgium). For BC, undiluted milk samples were used and automatically analyzed by means of a Bactoscan™ FC (Foss Electric, Hillerød, Denmark). Bacterial counts were expressed as the number of IBC/mL of milk. For CC, milk samples were first diluted using trypton salt broth at 1:10. One mL of diluted milk was plated on Petrifilm CC plates and incubated at 30°C for 24h. Colony-forming units were counted electronically using an automated colony counter (protoCOL, Synbiosis, Cambridge, UK). Coliform counts were expressed as the number of colony-forming units per milliliter of milk. For the freezing point, undiluted milk samples were used and automatically analyzed by means of a Milkoscan™ 4000. Visible impurities were determined by a filtration test. Briefly, 40 mL of undiluted milk was drawn through the watt disk with a special device (Ledoux b.v., Dodewaard, The Netherlands). After filtration, the watt disk was dried for 1h by room temperature and was checked for visible impurities. All deliveries were checked for residues with a Delvo test (DSM Food Specialties, Delft, the Netherlands) before processing the milk (Angelidis et al., 1999). Per month, a geometric mean SCC, BC and CC was calculated based on the recordings of the last 2 months for BC and CC and the last 3 months for BMSCC.

Definitions

Current Milk Quality Premium (MQP). Several criteria were required to achieve the premium during the study period. The herds had to be part of the Dairy Quality Assurance program for all months of the year 2009. Additionally, the farm had to meet all legal standards regarding the filtration test, freezing point and residues. Regarding the BC and BMSCC levels, the premium was achieved if the geometric mean BC was < 50 x 10^3 IBC/mL and the geometric mean CC was < 50 cfu/mL over the last two months (2 recordings per month) in combination with a geometric mean BMSCC < 350 x 10^3 cells/mL (4 recordings per month) over the last three months.

Hypothetical Milk Quality Premium (hMQP). A hypothetical premium would have been achieved if, based on the abovementioned standards, the level of BMSCC would have been lowered to a geometric mean BMSCC < 250 x 10^3 cells/mL rather than 350 x 10^3 cells/mL, with all other legal standards remaining unchanged, for all months of the year 2009. So, in this system we would not use CC as a standard.

Statistical Analyses

All data were entered in an electronic spreadsheet program (Excel 2010, Microsoft Corporation) and were checked for unlikely values. Monthly geometric means for SCC (n = 2904), BC (n = 2904) and CC (n = 2904) were available for 242 farms. As well, monthly test results of freezing point (n = 2904), residues (n = 2904) and filtration test (n = 2904) were available.

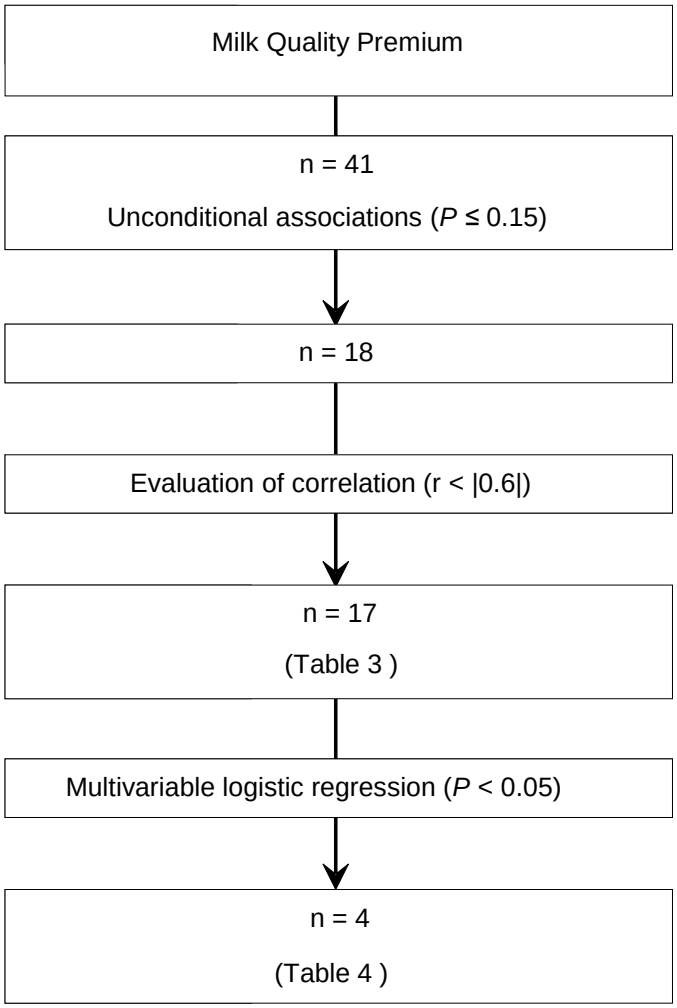


Figure 1. Flow chart of variable reduction through different steps in the statistical analysis.

The regression model-building process to identify management practices associated with achieving MQP involved several steps as described previously (De Vliegher et al., 2004) and is presented in a flowchart (Figure 1). A binary logistic regression model with MPQ as dependent variable was fit using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

Table 1. Overview of all herd management practices collected via a web-based questionnaire on 242 dairy herds in Belgium.

General management	
	Type of livestock farming, expected time the farm will still exist, herd size, number of lactating cows, milk quota size, duration of access to pasture during summer, barn type, cleaning frequency of the housing
Herd health management	
	Registration of animal diseases, herd health monitoring by veterinarian, participation in udder health monitoring, monthly estimated incidence of clinical mastitis, antimicrobial treatment during lactation of subclinical mastitis, treatment of prepartum heifers with antimicrobials, treatment based on vet advice or culture results, treatment duration of mild clinical mastitis, use of self-prepared off-label udder infusions
Milking management	
	Milking machine type, milking parlor type, cows kept in headlock after milking, use of automatic cluster removal, providing a preparation lag-time of 60 s, teat preparation method, application of premilking teat disinfection, application of postmilking teat disinfection, type of machine unit liner, rinsing of machine unit liners, replacement of machine unit liners
Calving management	
	Calving on pasture, presence of calving pen, use of calving pen for sick cows
Nutrition management	
	Concentrate provided during milking, concentrate provided on top of forage, concentrate provided via total mixed ration, concentrate provided via automatic feeder, forage provided, type of forage feeding system
Dry cow management	
	Drying-off procedure, use of long-acting antimicrobials, adapted diet provided, mineral/vitamin mix provided, use of external teat sealer, use of internal teat sealer

Initially, unconditional associations were tested between the binary dependent variable MQP at the herd level and all management practices (n = 41, Table 1). Statistical significance in this step was assessed at $P < 0.15$. Some of the categorical variables were recoded based on biological relatedness because of low frequencies in one or more categories in this step. Second, Pearson and Spearman's rank correlation coefficients were calculated among the significant independent variables to avoid multicollinearity in the next steps. If two independent variables had a correlation coefficient ≥ |0.6|, only the one with the highest statistical significance or the most relevant variable was selected for further analysis. In the third step, a multivariable model was built with the remaining management variables as independent variables, and with MQP as dependent variable (Table 1). Non-significant variables were removed using backwards elimination at $P \leq 0.05$. All first-order interactions between the

remaining variables in the multivariable model were tested and removed when non-significant (Wald's tests, $P > 0.05$).

Results

Descriptive Results

The average herd size of the 242 herds included in the study was 65.8 [interquartile range (IQR) from 45 to 80] lactating cows per herd, with an average milk quota size of 552,188 kg of milk (IQR from 375,750 to 676,500) per year. The average of the BMSCC was 202,000 cells/mL (IQR from 155,000 to 239,800 cells/mL). The median of the BC and CC was 7.0 x 10^3 IBC/mL (IQR from 5 x 103 to 11 x 10^3 IBC/mL) and 4.0 cfu/mL (IQR from 2 to 12 cfu/mL), respectively. Averages of the BC and CC were 8.9 x 10^3 IBC/mL and 13 cfu/mL, respectively.

Of the 242 herds, 94.6% (n = 229) used a conventional milking system, whereas 5.4% (n=13) were milking their cows with an automated milking system (AMS). Eighty percent of the farms (n = 194) housed their cows in a freestall with slatted floor. In almost 60% of the herds (n = 138), some herd health aspects such as fertility, and (or) udder health, and (or) heifer rearing, and (or) claw health were monitored by a veterinarian on a regular basis. Disease registration was systematically done on approximately half of the herds. More than 80% (n = 198) of the farmers claimed their farm would still exist for more than 10 years.

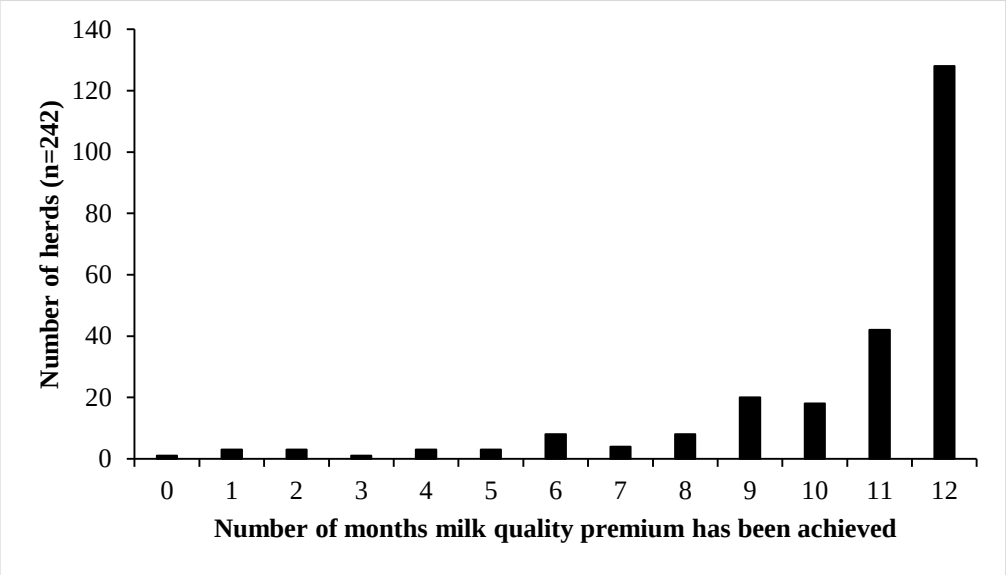
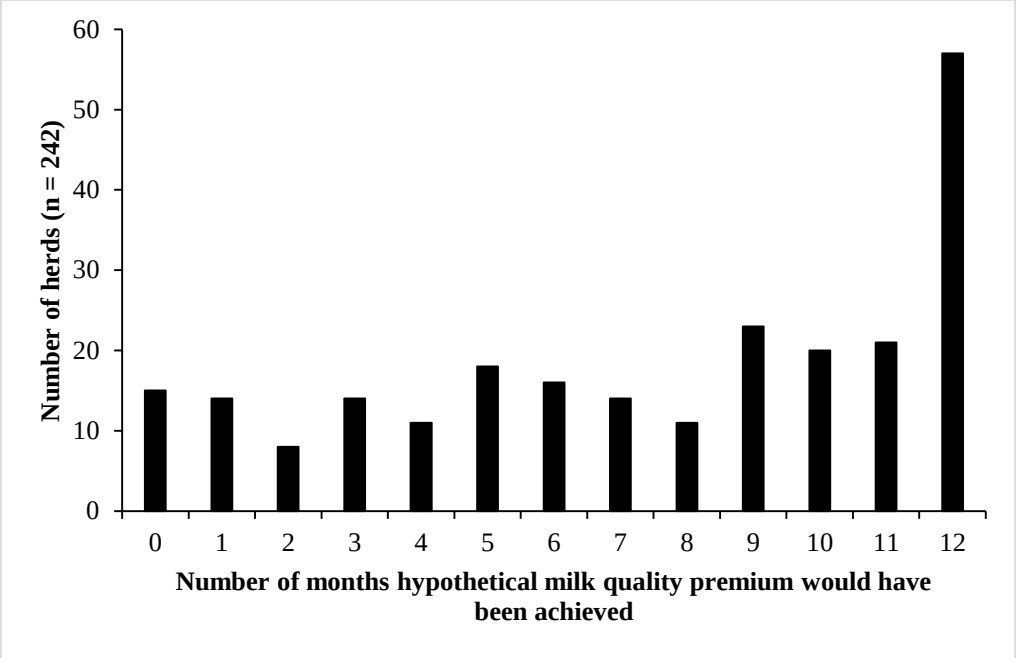


Figure 2. Distribution of the herds (n = 242) that achieved the milk quality premium in 2009.

Only 52.9% (n = 128) of the herds achieved their milk quality premium for all 12 months in 2009 (Figure 2). If the BMSCC threshold would have been 250 x 10^3 cells/mL, only 23.5% (n = 57) would have achieved their milk quality premium in all months of 2009 (Figure 3).



[1] Bulk milk somatic cell count (BMSCC) level was set at 250,000 cells/mL and all other standards remained unchanged.

Figure 3. Distribution of the herds (n = 242) that would have achieved a hypothetical milk quality premium[1] in 2009.

The main reasons for not achieving the milk quality premiums in 2009 are listed in Table 2a. For 406 months, the MQP was not achieved, but in 47 cases, the MQP could not be achieved due to more than one reason, resulting in a combined total of 359 months that the MQP was not achieved. In the current premium system, CC is the predominant reason (63.3% of the reasons for not achieving the premium) for not achieving the monthly milk quality premium, whereas this would be due to BMSCC (77.2% of the reasons for not achieving the premium) if the premium system would have been changed using a lower 250 x 10^3 cells/mL BMSCC threshold (Table 2b). Also in the hMQP, for 1280 months the hMQP would not have been achieved, but in 175 cases a hMQP would not be achieved due to more than one reason, resulting in a combined total of 1105 months the hMQP would not be achieved. Average BMSCC, BC and CC of herds achieving and not achieving the MQP and hMQP are listed in Table 3a and Table 3b.

Table 2a. Reasons for not achieving the monthly milk quality premium in 2009.

	Milk quality premium		
Reason	Not achieved (months)	Achieved (months)	% not achieved
Somatic cell count	114	2790	3.9
Bacterial count	19	2885	0.7
Coliform count	257	2647	8.8
Filtration	0	2904	0.0
Freezing point	4	2900	0.1
Residu	12	2892	0.4
Combined total	359	2545	12.36

Table 2b. Reasons for not achieving the monthly hypothetical milk quality premium in 2009.

	Hypothetical milk quality premium		
Reason	Not achieved (months)	Achieved (months)	% not achieved
Somatic cell count	988	1916	34.0
Bacterial count	19	2885	0.7
Coliform count	257	2647	8.8
Filtration	0	2904	0.0
Freezing point	4	2900	0.1
Residu	12	2892	0.4
Combined total	1105	1799	38.05

Table 3a. Average bulk milk somatic cell count (BMSCC), bacterial count (BC) and coliform count (CC) of herds achieving and not achieving the monthly milk quality premium.

	Milk quality premium	
Variable	Achieved	Not achieved
BMSCC (cells/mL)	180,000	226,000
BC (x10^3 IBC/mL)	7.5	10.3
CC (cfu/mL)	7.24	19.4

Table 3b. Average bulk milk somatic cell count (BMSCC), bacterial count (BC) and coliform count (CC) of herds achieving and not achieving the monthly hypothetical milk quality premium.

	Hypothetical milk quality premium	
Variable	Achieved	Not achieved
BMSCC (cells/mL)	151,000	217,000
BC (x10^3 IBC/mL)	6.4	9.6
CC (cfu/mL)	5.7	15.3

Table 4. Unconditional associations for achieving milk quality premium (MQP) ($P < 0.15$).

	Milk quality premium				
Independent variables	**Herds (n)**	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
General management					
Calving on pasture					0.041
No	67	Ref.	…		
Some/all	175	0.591	0.290	1.807	
Concentrates during milking provided					0.111
No	211	Ref.	…		
Yes	31	-0.621	0.390	0.537	
Number of lactating cows					
Number of cows	242	-0.003	0.002	0.997	0.099
Calving pen used as sick pen					0.142
No	36	Ref.	…		
No calving pen	135	-0.251	0.411	0.778	
Yes	71	0.408	0.296	1.504	
Herd health management					
Participation in veterinary herd health program					0.145
No	104	Ref.	…		
Yes	138	0.381	0.262	1.464	
Estimated incidence of clinical mastitis in 2009					0.012
≤ 3 %	116	Ref.	…		
> 3 %	126	-0.662	0.263	0.516	
Milking management					
Type of milking machine					0.008
Low-line set-up	181	Ref.	…		
High-line set-up	20	-1.505	0.493	0.348	
With reservoir	28	-0.150	0.411	0.861	
Automated milking system	13	-2.923	1.052	0.054	

Table 4. (continued).

	Milk quality premium				
Independent variables	**Herds (n)**	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Milking parlor					0.083
Fishbone	140	Ref.	…		
Tandem	51	-0.287	0.331	0.750	
Rotary	17	-0.366	0.516	0.693	
Side-by-side	5	-0.079	0.929	0.924	
Automatic	13	-2.969	1.055	0.051	
Tie stall	16	-0.735	0.530	0.479	
Automated milking system					0.007
No	229	Ref.	…		
Yes	13	-2.811	1.049	0.060	
Use of automatic cluster removal					0.111
No	35	Ref.	…		
Yes	207	0.589	0.369	1.803	
On-time replacement of machine unit liners					0.074
No	216	Ref.	…		
Yes	26	-0.760	0.426	0.467	
Rinsing of machine unit liners after milking					0.104
No	200	Ref.	…		
Yes, high SCC cows with hot water or disinfectants	42	0.602	0.370	1.826	
Teat preparation					0.054
Paper towel	13	Ref.	…		
No	43	-2.753	1.054	0.064	
Cloths	9	-0.035	0.348	0.966	
Disinfection towels	25	-0.045	0.691	0.956	
Wet and dry after / automatic	152	0.676	0.475	1.966	

Table 4. (continued).

	Milk quality premium				
Independent variables	**Herds (n)**	**Beta**	**SE**[1]	**OR**[2]	***P*-value**
Providing preparation lag time of 60 s					0.038
Never / rarely / sometimes	88	Ref.	…		
Not applicable	19	-1.258	0.563	0.284	
Always / usually	135	0.146	0.277	1.158	
Application of premilking teat disinfection					0.048
Never / rarely / sometimes	218	Ref.	…		
Always / usually	24	0.970	0.491	2.638	
Dry cow management					
Adapted diet provided					0.076
No	40	Ref.	…		
Yes	202	0.622	0.350	1.862	
Mineral/vitamin mix provided to dry cows					0.030
No	64	Ref.	…		
Yes	178	0.638	0.295	1.893	
Drying-off procedure					0.136
Intermittent milking	70	Ref.	…		
Abrupt	172	-0.433	0.291	0.649	

[1] SE = Standard error, [2] OR = Odds ratio.

Unconditional Associations

A first reduction based on unconditional associations and highly correlated variables revealed 18 herd management practices to be associated ($P \leq 0.15$) with achieving MQP (Table 4). A strong correlation was present between the number of lactating cows and the quota size. The number of lactating cows was selected for further analysis because this variable is more precise to describe a farm, since some farmers were not using their quota size as a goal for milk production.

Table 5. Final multivariable model for achieving milk quality premium (MQP) ($P<0.05$).

	Milk quality premium			
Independent variables	**Beta**	**SE[1]**	**OR[2]**	***P*-value**
Premilking teat disinfection				0.049
no	Ref.	…		
yes	1.002	0.555	2.981	
Dry cows received minerals				0.031
no	Ref.	…		
yes	0.669	0.309	1.951	
Estimated incidence of clinical mastitis in 2009				0.007
≤3%	Ref.	…		
>3%	-0.756	0.278	0.470	
Automated milking system				0.003
no	Ref.	…		
yes	-3.132	1.064	0.044	

[1] SE = Standard error, [2] OR = Odds ratio

Final Multivariable Model

The final multivariable model for achieving MQP is presented in Table 5. The final model revealed that herds of where application of premilking teat desinfection was done, were significantly more likely to achieve the MQP. Also, when dry cows received minerals, herds were more likely to achieve the MQP. On the other hand, herds with a high estimated incidence of clinical mastitis (>3%) and herds with an AMS were significantly less likely to achieve the MQP in 2009.

Discussion

To determine what management factors were associated with achieving MQP in Flanders, a web-based questionnaire was used as this allowed for relatively easy, efficient and cheap data collection. The response rate was around 24%, which represented 4% of the dairy farmers in Flanders in 2009 (Annual Report 2009, Belgian Dairy Confederation, Leuven, Belgium). The average herd and quota size of the selected farms were slightly higher than the Flemish average, being 44 lactating cows and 310,650 kg of milk per herd, respectively (Annual Report 2009, Belgian Dairy Confederation, Leuven, Belgium). Herd size and the need to have internet access, might have selected the input of more contemporary farmers that are also better managers, potentially diminishing the external validity of the data (Dohoo et al., 2009). Still, the high variation in BMSCC between the respondents suggests a wide diversity of management styles (Barkema et al., 1998). Also, both the average BMSCC and BC of the selected herds approached the average BMSCC and BC for the whole of Flanders being 230,000 cells/mL and 11.3 x 10^3 IBC/mL, respectively, in 2009 (Annual Report 2009, Milk Control Center Flanders, Lier, Belgium). The average CC of the participating herds was even higher than the Flemish average of 10 cfu/mL in that period (Annual Report 2009, Milk Control Center Flanders, Lier, Belgium).

The finding that only 52.9% of the herds achieved their MQP 12 months in 2009 was surprising, especially because of the fact that the thresholds for the legal standards and for achieving the premium are close together. On the other hand, premiums only represent 3% of the milk price, which is low compared to other premium systems in the EU. Most of the milk buyers in the EU pay a premium of 3-5% of the milk price below the premium threshold, a neutral price above that premium threshold and then introduce price reductions of 5-10% from a higher threshold on, often 250 x 10^3 cells/mL up to the regulatory level (Hillerton and Berry, 2004). However, as shown by Valeeva et al. (2007), with respect to monetary factors, farmers are expected to be more motivated by a quality penalty system design than by a quality premium system design, which could potentially explain at least partly why only half of the herds achieved their milk quality premium.

Interestingly, CC was the main reason for not achieving the MQP in Flanders, but if the threshold for BMSCC would have been 250 x 10^3 cells/mL then BMSCC (77.2%) would have been the main reason. However, the median and average CC in our study were much lower than the average CC of 44 cfu/mL across a number of studies (Elmoslemany et al., 2009; Pantoja et al., 2011), still suggesting better conditions concerning milk refrigeration, milking machine sanitation and premilking hygiene on the Flemish dairy farms compared to the farms

included in the other studies (Jayarao and Wolfgang, 2003). Although there is no regulatory limit on the amount of coliforms that can be present in raw milk, the Grade "A" Pasteurized Milk Ordinance (Food and Drug Administration, 2009) requires pasteurized milk to have a CC ≤ 10 cfu/mL. Other research has used ≤50 cfu/mL as the proposed cutoff for "good quality" in regard to coliforms in raw milk (Jayarao et al., 2004), which is in agreement with the standards for achieving the MQP in Flanders.

The implementation of CC in a premium system as is the case in Flanders is quite exceptional. As shown in Table 3a, this current premium system selects for low BMSCC, BC and CC. If CC would not have been taken into account, then BMSCC, BC and CC would have been 357 x 10^3 cells/mL, 21.9 x 10^3 IBC/mL and 70.8 cfu/mL, respectively, in the farms that did not achieve the premium for the whole year. In contrast, farms that would achieve the premium in a system without CC, BMSCC, BC and CC would have 216 x 10^3 cells/mL, 8.07 x 10^3 IBC/mL and 18.8 cfu/mL, respectively.

There are several possible reasons why CC was the main cause of not achieving MQP. First, in contrast with BMSCC, CC is only measured twice a month, which makes it more difficult to react as a farmer when a high CC is observed. Second, use of geometric means is useful to correct for outliers when the data is skewed. Coliform count and BC are positively skewed and also the distribution of the geometric means did not normalize the data. Also, the geometric mean BMSCC was calculated based on the recordings of the last 3 months, whereas this was the last 2 months for BC and CC. Third, in contrast to BC, CC is measured after dilution (1:10), making incorrect counts more likely.

According to Schukken et al. (1990), the annual mean BMSCC should be lower than 250 x 10^3 cells/mL to minimize the chance of passing the penalty limit of 400 x 10^3 cells/mL in any given bulk tank. Using a threshold of 250 x 10^3 cells/mL rather than 350 x 10^3 cells/mL in a hMQP system, would have most likely selected for a lower BC and CC as well. However, we should be cautious to conclude this, since the data only reflect the management during 2009 from farmers who were familiar with the milk quality system and not aware of the hypothetical quality system. Because BMSCC is measured four times a month, it is normally distributed and the geometric mean is calculated based on the last 3 months, the authors would prefer the hMQP system over the current MQP system. Also, only a small proportion of the variation in CC can be explained by manageable practices different from those related to milking and equipment hygiene (Piepers et al., 2014), whereas Pantoja et al. (2009) mentioned that every 10 x 10^3 cells/mL increase in BMSCC increased the odds of elevated BC and CC by 2.4 and 4.3%, respectively.

Four variables reflecting milking, herd health and dry cow management were significantly associated with achieving MQP during 2009 in Flanders. Obviously, the fact that associations were statistically significant in the analyses not necessarily indicates that a causal relation exists (Dohoo et al., 1997). Also, when no variation is present in the dataset, it doesn't mean that there is no association between the studied management practices and the outcome variable(s). In this study, well-known management practices for good udder health and milk quality such as postmilking teat disinfection and changing liners in time were done respectively, by more than 90% and less than 10% of the herds, making it difficult to show an association. The use of an AMS decreased the likelihood of achieving the MQP the whole year. This is in line with the report from The Netherlands indicating that the BC, SCC and freezing point may be altered by the introduction of AMS (Jepsen and Rasmussen, 2000; Klungel et al., 1998; 2000; Vorst and Hogeveen, 2000). Also, Hamann and Zecconi (1998) concluded that electrical conductivity does not identify mastitic quarters or cows with sufficient accuracy, and the current generation of AMS does not sort milk according to the appearance of the foremilk (Rasmussen et al., 2002), making poor milk quality in the bulk tank more likely. The finding that herds where dry cows were fed with minerals were more likely to achieve the MQP was in line with the results of Piepers et al. (2014), who used the same dataset as ours and demonstrated that providing a mineral/vitamin mix to dry cows was negatively associated with BC. The latter could be a reflection of a relationship between MQP and BC. Supplementing minerals during dry period could be helpful in curing existing IMI causing subclinical mastitis. The use of minerals could also be a confounder and could simply be a reflection of the farmers' attitude towards the importance of appropriate dry cow feeding. Achieving MQP was also associated with premilking teat disinfection which was not that unexpected as Elmoslemany et al. (2009) reported that the risk of having high BC increased by 5.3 for each unit increase in the teat-end cleanliness before the milking unit was attached. Our finding corresponds well to the numerous studies that showed a strong teat microbial load reducing effect of premilking teat disinfection (e.g. Galton et al., 1986; Magnusson et al., 2006; Gibson et al., 2008) and on reducing aerobic mesophilic bacteria (Oliveira et al., 2011) and BC (Piepers et al., 2014).

According to Dohoo and Leslie (1991), SCC is mainly determined by IMI and is therefore an excellent proxy to measure prevalence and even incidence of IMI whether clinical signs of mastitis are present or not. This probably explains the fact that herds with a high estimated incidence of clinical mastitis (>3% cases per month) were less likely to achieve the MQP. However, some studies suggested that low BMSCC was associated with high IRCM (Elbers et al., 1998; Beaudeau et al., 2002; Green et al., 2004) whereas other work did not reveal any relationship (Barkema et al., 1998; Beaudeau et al., 1998). Also, herds having a higher

prevalence/incidence of (clinical) mastitis are using more antimicrobials (Stevens et al., 2016) resulting in a higher chance of detecting residues in the bulk tank. Actually, violation against antimicrobial residues in the bulk tank was more often observed (0.46%) in herds with a high estimated incidence of clinical mastitis (> 3%) (n = 7) compared to herds with a low estimated incidence of clinical mastitis (≤ 3%) (n = 5) (0.36%). However, we should be careful in interpreting the incidence data since the majority of participating farmers admitted not to keep disease records, hence the use of the term “estimated” incidence.

Conclusions

Only half of the herds achieved their milk quality premium in all 12 months in 2009, suggesting that there is still room for opitmization of the profitability on dairy herds in Flanders by improving the udder health and milk quality. If the BMSCC threshold would have been 250 x 10^3 rather than 350 x 10^3 cells/mL in a hypothetical system, this would have further reduced to 23.5%. The main reason for not achieving the milk quality premiums in the current system was CC, whereas this would have been BMSCC in the hMQP system. The latter implies that in the current system achieving the milk quality premium is most probably more determined by factors related to milking and equipment hygiene and milk storage conditions than by factors related to either herd health, transition, feeding and housing management. Still, application of premilking teat disinfection, providing a mineral/vitamin mix to the dry cows received minerals, and reducing the incidence of clinical mastitis might be helpful in achieving the milk quality premium.

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References

Angelidis A. S, T. B. Farver , and J. S. Cullor. 1999. Evaluation of the Delvo-X-Press Assay for Detecting Antibiotic Residues in Milk Samples from Individual Cows. J. Food. Prot. 62:1183-1190.

Barbano, D. M., Y. Ma, and M. V. Santos. 2006. Influence of raw milk quality on fluid milk shelf life. J. Dairy Sci. 89 suppl.1:15-19.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, G. Benedictus, and A. Brand. 1998. Management practices associated with low, medium, and high somatic cell counts in bulk milk. J. Dairy Sci. 81:1917-1927.

Beaudeau, F., H. Seegers, C. Fourichon, and P. Hortet. 1998. Association between milk somatic cell counts up to 400,000 cells/mL and clinical mastitis in French Holstein cows. Vet. Rec. 143:685-687.

Beaudeau, F., C. Fourichon, H. Seegers, and N. Bareille. 2002. Risk of clinical mastitis in dairy herds with a high proportion of low individual milk somatic cell-count. Prev. Vet. Med. 53:43-54.

Belgian Dairy Confederation. 2009. Annual report. Belgian Dairy Confederation, Leuven, Belgium.

Berry, D. P., B. O'Brien, E. J. O'Callaghan, K. O. Sullivan, and W. J. Meaney. 2006. Temporal trends in bulk tank somatic cell count and total bacterial count in Irish dairy herds during the past decade. J. Dairy Sci. 89:4083-4093.

Dohoo, I. R., and K. E. Leslie. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10:225-237.

Dohoo, I. R., C. Ducrot, C. Fourichon, A. Donald, and D. Hurnik. 1997. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29:221-239.

Dohoo, I., M. Wayne, and H. Stryhn (Eds.), 2009. Questionnaire design. In: Veterinary Epidemiologic Research 2nd Edition, VER Incorporated, Charlottetown, Canada p. 58-72.

Elbers, A. R. W., J. D. Miltenburg, D. De Lange, A. P. P. Crauwels, H. W. Barkema, and Y. H. Schukken. 1998. Risk factors for clinical mastitis in a random sample of dairy herds from the southern part of The Netherlands. J. Dairy Sci. 81:420-426.

Elmoslemany, A. M., G. P. Keefe, I. R. Dohoo, and B. M. Jayarao. 2009. Risk factors for bacteriological quality of bulk tank milk in Prince Edwards Island dairy herds. Part 2: Bacteria count-specific risk factors. J. Dairy Sci. 92:2644-2652.

FDA. 2009. Grade "A" pasteurized milk ordinance, 2009 rev. Accessed May 15, 2015. http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM209789.pdf.

Flores-Miyamoto, A., M.W. Reij, and A.G.J Velthuis. 2014. Do farm audits improve milk quality? J. Dairy Sci. 97:1-12.

Galton, D. M., L. G. Petersson, and W. G. Merill. 1986. Effects of premilking udder preparation practices on bacterial counts in milk and on teats. J. Dairy Sci. 69:260-266.

Gibson, H., L. A. Sinclair, C. M. Brizuela, H. L. Worton, and R. G. Protheroe. 2008. Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. Lett. Appl. Microbiol. 46:295-300.

Green, M. J., P. R. Burton, L. E. Green, Y. H. Schukken, A. J. Bradley, E. J. Peeler, and G. F. Medley. 2004. The use of Markov chain Monte Carlo for analysis of correlated binary data: Patterns of somatic cells in milk and the risk of clinical mastitis in dairy cows. Prev. Vet. Med. 64:157-174.

Hamann, J., and A. Zecconi. 1998. Evaluation of the electrical conductivity of milk as a mastitis indicator. Bulletin of IDF No. 334, 26pp.

Hillerton, E., and E. A. Berry. 2004. Quality of the milk supply: European regulations versus practice. NMC Annual Meeting Proceedings 2004. p207-214

Hogan, J. 2005. Human health risks associated with high BTSCC milk: Symposium overview. Pages 73-75 in Proc. 44th NMC Annu. Mtg. Natl. Mastitis Counc. Inc., Verona, WI.

Jayarao, B. M., and D. R. Henning. 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci. 84:2157-2162.

Jayarao, B. M., and D. R. Wolfgang. 2003. Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health. Vet. Clin. North Am.: Food Anim. Pract. 19:75-92.

Jayarao, B. M., S. R. Pillai, A. A. Sawant, D. R. Wolfgang, and N. V. Hegde. 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87:3561-3573.

Jepsen, L., and M. D. Rasmussen. 2000. Milk quality on Danish farms with automatic milking systems. Natl. Mastitis Council Ann. Meet. 39:181-182.

Keefe, G. and A. Elmoslemany. 2007. Consumer acceptance of fluid milk after raw milk selection using bulk tank bacteriologic and somatic cell count criteria. NMC Annual Meeting Proceedings 2007, p218-219.

Klei, L., J. Yun, A. Sapru, J. Lynch, D. Barbano, P. Sears, and D. Galton. 1998. Effects of milk somatic cell count on cottage cheese yield and quality. J. Dairy Sci. 81:1205-1213.

Klungel, G. H., B. A. Slaghuis, and H. Hogeveen. 1998. Werk aan de robotwinkel. Melkkwaliteit bij automatisch melken verdient aandacht. Veeteelt, August, pp. 888-890.

Klungel, G. H., B. A. Slaghuis and H. Hogeveen. 2000. The effect of the introduction of automatic milking systems on milk quality. J. Dairy Sci. 83:1998-2003.

Magnusson, M., A. Christiansson, B. Svensson, and C. Kolstrup. 2006. Effect of different premilking manual teat-cleaning methods on bacterial spores in milk. J. Dairy Sci. 89: 3866-3875.

Milk Control Center Flanders. 2009. Annual Report. Milk Control Center Flanders, Lier, Belgium.

More, S.J. 2009. Global trends in milk quality: implications for the Irish dairy industry. Irish Vet. J. 62: 5-14.

Nightingale, C., K. Dhuyvetter, R. Mitchell, and Y.H. Schukken. 2008. Influence of variable milk quality premiums on observed milk quality. J. Dairy Sci. 91:1236-1244.

Oliveira, C. J. B., W. D. Lopes Junior, R. C. R. E. Queroga, P. E. N. Givisiez, P. S. Azevedo, W. E. Pereira, and W. A. Gebreyes. 2011. Risk factors associated with selected indicators of milk quality in semiarid northeastern. Brazil. J. Dairy Sci. 94:3166-3175.

Pantoja, J. C. F.,D. J. Reinemann, and P. L. Ruegg. 2009. Associations among milk quality indicators in raw bulk milk. J. Dairy Sci. 92:4978-4987.

Pantoja, J. C. F.,D. J. Reinemann, and P. L. Ruegg. 2011. Factors associated with coliform count in unpasteurized bulk milk. J. Dairy Sci. 94: 2680-2691.

Piepers, S., P. Zrimsek, P. Passchyn, and S. De Vliegher. 2014. Manageable risk factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds. J. Dairy Sci. 97:3409-3419.

Rasmussen, M. D., M. Bjerring, P. Justesen, and L. Jepsen. 2002. Milk quality on Danish farms with automatic milking systems. J. Dairy Sci. 85:2869-2878.

Ruegg, P. L., and T. J. Tabone. 2000. The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds. J. Dairy Sci. 83:2805-2809.

Saville, W. J., T. E. Wittum, and K. L. Smith. 2000. Association between measures of milk quality and risk of violative antimicrobial residues in grade-A raw milk. J. Am. Vet. Med. Assoc. 217:541-545.

Schukken, Y. H., F. J. Grommers, D. van de Geer, 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. 73:3463-3471.

Schukken, Y. H., K. E. Leslie, A. J. Weersink, and S. W. Martin. 1992. Ontario bulk milk somatic cell count reduction program. 1. Impact on somatic cell counts and milk quality. J. Dairy Sci. 75:3352-3358.

Stevens, M., S. Piepers, K. Supré, J. Dewulf, and S. De Vliegher. 2016. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. J. Dairy Sci. 99:2118-2130.

Straley, B. A., S. C. Donaldson, N. V. Hedge, A. A. Sawant, V. Srinivasan, S. P. Oliver, and B. M. Jayarao. 2006. Public health significance of antimicrobial-resistant gram-negative bacteria in raw bulk tank milk. Foodborne Pathog. Dis. 3:222-233.

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

van Schaik, G., M. Lotem, and Y. H. Schukken. 2002. Trends in somatic cell counts, bacterial counts, and antibiotic residue violations in New York State during 1999-2000. J. Dairy Sci. 85:782-789.

Vorst, Y. v.d., and H. Hogeveen. 2000. Pages 73-82 in Proc. Int. Symp. Robotic Milking. H. Hogeveen and A. Meijering, ed., Wageningen Pers, Lelystad, The Netherlands.

Veerkamp, R. F., A. W. Stott, W. G. Hill, and S. Brotherstone. 1998. The economic value of somatic cell count payment schemes for UK dairy cattle breeding programmes. Anim. Sci. 66:293-298.

Chapter 5

Management and Treatment of Heifer Mastitis

Chapter 5.1

Concentration of Penicillin G in Mammary Tissue and Secretion of End-term Dairy Heifers following Systemic Prepartum Administration of Penethamate Hydriodide

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Abstract

The aim of this study was to assess the concentration of penicillin G in mammary tissue and secretion of dry heifers following systemic administration of penethamate hydriodide. Six dairy heifers in late gestation received a single intramuscular injection of 10 g penethamate hydriodide and were sacrificed 24, 48 or 144 hours after treatment. Penicillin G concentrations were measured in mammary tissue and secretion samples using high performance liquid chromatography. Penicillin G was detected in the udder of two animals euthanized at 24 hours (mammary tissue and secretion) and at 48 hours post treatment (mammary secretion only) after administration at concentrations still close to or above MIC90 values reported for the pathogens associated with heifer mastitis. Antibiotic concentration shortly after administration will have been substantially higher indicating a potential for systemic treatment with penethamate hydriodide to control prepartum intramammary infections in heifers without the disadvantages of local therapy such as teat contamination or risk of trauma for the administrator.

Introduction

For many years there have been reports that a large proportion of dairy heifers calve with infected quarters (Fox, 2009). This condition has been referred to as heifer mastitis and studies have shown a wide variation in prevalence, from 74.6 to 29.0% (Trinidad et al., 1990, Oliver and Mitchell, 1983) and from 55.0 to 12.3% (Roberson et al., 1994, Parker et al., 2007) of quarters being reported as culture positive before and at calving, respectively. A number of pathogens have been isolated but studies have shown that infections are predominantly due to Gram-positive pathogens, specifically coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, and environmental streptococci (Fox, 2009).

Heifer mastitis can have a negative impact on future productive life (De Vliegher et al., 2004, 2005a, 2005b), the effect depending on factors such as virulence of the pathogens involved and time of onset of the intramammary infection (IMI) (Piepers et al., 2009). The cost of heifer mastitis in early lactation on an average Dutch/Flemish dairy farm has recently been estimated to vary from €4 to €82 per heifer with an average of €31 (Huijps et al., 2009).

To address this issue, antibiotic treatments have been used empirically before calving. Various studies have shown that the use of dry or lactating cow intramammary products prior to calving in heifers can be beneficial in reducing levels of mastitis pathogens isolated post partum and in increasing milk yield during lactation (Nickerson, 2009). Systemic rather than local antibiotic treatment presents the advantages of decreased risk of teat contamination, more convenient and safer to administer, and four quarters being treated with a single administration.

Penethamate hydriodide is a prodrug which releases penicillin G on hydrolysis. It easily crosses the blood–milk barrier and concentrates in udder tissues and milk after intramuscular (IM) administration to lactating cows (Ziv, 1980) The in-vitro spectrum of activity is mainly within the Gram-positive class of bacteria e.g. *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus* spp. A recent study on a herd struggling with *Staphylococcus aureus* infections showed that IM treatment of heifers at parturition with penethamate hydriodide prevented IMI during the first week post partum and resulted in a significant increase in milk yield (Kreiger et al., 2007). Another study on a 350 cow dairy herd having problems with *S. aureus* infections showed a significant reduction in the number of IMI after introduction of a regimen to inject heifers 2 months prior to calving with penethamate hydriodide intramuscularly (Moroni et al., 2002). A prerequisite for the successful use of this treatment in heifers and cows before calving is widespread distribution of the drug in the dry udder.

The aim of this study was to assess the concentration of penicillin G in mammary tissue and secretion of heifers in the third trimester of pregnancy following a single IM injection of penethamate hydriodide.

Materials and Methods

Animals

The study group consisted of 6 clinically healthy primiparous dairy heifers (4 Montbeliarde and 2 cross-breds Montbeliarde x Charolais) in their 8th to 9th month of gestation, weighing from 540 to 630 kg (average 600 kg) and aged between 2.5 and 3.5 years (average 2.6 years). Animals had received no treatment with veterinary drugs for at least 2 weeks prior to the beginning of a 7 day acclimatization period.

Drug administration

After the acclimatization period each of the 6 animals was treated by deep IM injection with one vial of the reconstituted test item containing 7.72 g penethamate hydriodide (Mammyzine®, Mamyzin®, STOP M®, Boehringer Ingelheim Vetmedica). This equated to doses ranging from 12.3 to 14.3 mg/kg. All animals were treated, once only, on the same day. No adverse reactions were noted.

Tissue and secretion samples

Animals were slaughtered randomly in groups of two at approximately 24, 48 and 144 hours post treatment. On the day of slaughter 100-120 g pieces of mammary tissue were removed from each quarter and individually minced before being placed in plastic bottles. Mammary secretions were obtained manually by pressing the mammary tissue of each quarter and collecting the liquid in plastic bottles. All the prepared samples were frozen within 2 to 3 hours, and stored at a temperature below -75°C until their analysis.

Penicillin G assay

The determination of penicillin G in mammary tissue and secretion was carried out using reverse phase high performance liquid chromatography (HPLC) based on Tarbin et al. (1995). Samples weighing 5 g of either mammary tissue (homogenised with 10mL water) or secretion (diluted with 10mL saturated dibasic sodium phosphate) were acidified with 0.17M sulphuric acid and protein precipitated by adding 5% sodium tungstate solution. The resulting mixture was sonicated, shaken for 30 min (horizontal shaker) and the aqueous layer recovered following centrifugation (12°C, 10 minutes, 2,800 g). A second extraction step followed, the second aqueous layer was combined with the first, and a 20% solution of sodium chloride

added. Mammary secretion extracts were adjusted to pH 8 using concentrated sodium hydroxide. Extracts obtained were then deposited on an SPE C18 cartridge (Waters, Milford, MA, USA) previously washed with methanol, water and a 2% sodium chloride solution. The cartridge was then washed with 2% sodium chloride solution followed by water, and dried. The cartridge was eluted with a mixture of acetonitrile, water and phosphate buffer. A derivatization mixture (1,2,4-triazol dissolved in water with 0.02 M mercury chloride, pH adjusted to 9.0) was added to the eluted sample and the mixture was placed in a water bath at 65°C for 2 hours. After cooling at room temperature and centrifugation (5 min, 2,800 g), 150 µL of the supernatant was injected in the HPLC system (Waters, Milford, MA, USA). The analyses were performed at 325 nm with a Spherisorb ODS2 column 250 mm x 4,6 mm (Waters, Milford, MA, USA) attached to an appropriate guard column filled with the same material. The analyses were carried out at room temperature. The mobile phase consisted of acetonitrile/0.1 M phosphate buffer containing 0.0157 M sodium thiosulfate (25/75, v/v). The retention time for penicillin G was ca. 12 min using an isocratic elution at 1 mL/min.

Calibration curves for penicillin G concentration in mammary tissue/secretion were linear between 50 and 1000 µg/kg ($r > 0.99$). The limit of quantification (LOQ) was set to 50 µg/kg for the two matrices and the limit of detection (LOD) was 18 µg/kg and 20 µg/kg for mammary tissue and mammary secretion, respectively. Penicillin G remained stable in extract for 24 hours (mammary secretion) or 48 hours (mammary tissue) at ambient temperature, and for 29 days (mammary tissue) or 90 days (mammary secretion) below -75°C, i.e. for storage periods longer than those used in the study.

Results

Mammary tissue

Penicillin G was detected at quantifiable levels in mammary tissue from the 8 quarters of the 2 animals sacrificed at 24 hours after treatment (ranging from 90.69 to 151.16 µg/kg) (Table 1). No major differences were seen between front and rear quarters. Penicillin G was not detected in mammary tissue from any of the quarters of the 4 animals euthanized at 2 and 6 days post treatment.

Mammary secretions

Quantifiable concentrations of penicillin G were detected in mammary secretion from all quarters of the 2 animals sacrificed on the first day after treatment (ranging from 74.53 to 291.31 µg/kg) (Table 1). Penicillin G concentrations were either similar to the ones observed in mammary tissue (animal 2, average 111.2 and 111.9 µg/kg in secretion and tissue, respectively), or approximately twice the concentrations in mammary tissue (animal 4, average 266.2 and 119.5 µg/kg in secretion and tissue, respectively). In animals euthanized on the second day after treatment, quantifiable concentrations of penicillin G were present in mammary secretions from the 4 quarters of animal 3, and from the rear quarters of animal 5. In animals sacrificed on the sixth day after treatment quantifiable concentrations of penicillin G were not detected in secretion samples from 7 of the 8 quarters. Penicillin G was detected at a low level in mammary secretion from the remaining quarter at a value close to the LOQ of 50 µg/kg.

Table 1. Penicillin G concentrations (µg/kg) in mammary tissue and secretion of - end-term dairy heifers following administration of penethamate hydriodide.

Time of sacrifice after administration	Animal number (month gestation)	Quarter position[1]	Mammary tissue	Mammary secretion
1 day (24 ± 0.5 hours)	2 (8th)	RL	96.49	74.53
		RR	109.47	98.67
		FL	139.18	119.18
		FR	102.61	152.42
	4 (9th)	RL	151.16	291.31
		RR	90.69	290.32
		FL	133.32	256.78
		FR	102.98	226.52
2 days (48 ± 0.5 hours)	3 (9th)	RL	< LOD[2]	77.39
		RR	< LOD	63.81
		FL	< LOD	81.76
		FR	< LOD	69.25
	5 (9th)	RL	< LOD	66.31
		RR	< LOD	73.97
		FL	< LOD	< LOQ[3]
		FR	< LOD	< LOQ
6 days (144 ± 0.5 hours)	1 (9th)	RL	< LOD	< LOQ
		RR	< LOD	< LOQ
		FL	< LOD	< LOQ
		FR	< LOD	< LOQ
	6 (9th)	RL	< LOD	74.62
		RR	< LOD	< LOQ
		FL	< LOD	< LOQ
		FR	< LOD	< LOQ

[1]RL: rear left, RR: rear right, FL: front left, FR: front right, [2]Level of detection (for tissue and secretion= 18 and 20 µg/kg, respectively), [3]Level of quantification (level of quantification = 50 µg/kg).

Discussion

Penicillin G reached tissues from all quarters of the udder of non-lactating heifers in high concentrations. Assuming the density of milk secretion and mammary tissues is higher than that of milk and colostrum, it appears that antibiotic concentrations present in the udder 24 hours after administration of penethamate hydriodide were near or above the MIC90 of penicillin for *S. aureus* (0.125->100 µg/ml) and Streptococci (0.07-2 µg/ml) as summarised by Erskine et al. (2004) and CNS (e.g. Gentilini et al., 2002, 4.4 µg/ml) i.e. the target pathogens commonly associated with heifer mastitis. Previous work shows that after a single IM injection of penethamate hydriodide in lactating cows the mean maximum penicillin G concentration is reached after 3.76 hours in plasma and after 5.91 hours in milk. With a mean half life of 4.27 hours (plasma) and 4.00 hours (milk) the maximum concentrations are at least 5 times those recorded at 24 hours post administration (Friton et al., 2003). This supports the view that in the current study penicillin G concentrations well above the MIC90 for the more common mastitis pathogens associated with heifer mastitis will have been reached.

Previous studies have shown that IM administration of penethamate hydriodide for 3 days can be efficacious in the treatment of clinical and subclinical mastitis in lactating cows (Salat et al., 2008; Serieys et al., 2005). Additionally, in another study where heifers were treated intramuscularly with penethamate hydriodide on one occasion 7 days prior to calving, periparturient mastitis incidence was 22% in treated heifers versus 46% in non-treated control heifers (Bryan and Friton, 2004).

Conclusions

We conclude that systemic use of penethamate hydriodide prior to calving can result in levels of penicillin G in mammary tissue and secretion substantially higher than the MIC90 of pathogens associated with heifer mastitis. These findings support the view that penethamate hydriodide administered via the IM route to heifers prior to calving could be an appropriate, although temporary, therapeutic choice while preventive measures are being implemented by the herd manager/farmer. Systemic treatment of end-term heifers that have never been constrained before has obvious advantages over local therapy using lactating or dry cow products. However, this therapeutic approach needs to be verified under field conditions to quantify the short and long term effects on udder health (somatic cell counts and clinical mastitis cases), and milk production.

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References

Bryan MA & Friton GM 2005 Stochastic economic modelling of the use of penethamate hydriodide (Mamyzin®). Proceedings of the 4th IDF International Mastitis Conference, Maastricht, The Netherlands 232-234.

De Vliegher S, Barkema HW, Stryhn H, Opsomer G & de Kruif A 2004 Impact of early lactation somatic cell count in heifers on somatic cell counts over the first lactation. Journal of Dairy Science 87 3672-3682.

De Vliegher S, Barkema HW, Opsomer G, de Kruif A & Duchateau L 2005a Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. Journal of Dairy Science 88 560-568.

De Vliegher S, Barkema HW, Stryhn H, Opsomer G & de Kruif A 2005b Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. Journal of Dairy Science 88 938-947.

Erskine R, Cullor J, Schallibaum M, Yancey B & Zecconi A 2004. Bovine mastitis pathogens and trends in resistance to antimicrobial drugs. Proceedings of the 43rd NMC meeting, Charlotte, North Carolina, US 400-414.

Friton G, Van Hattum JJC & Hörstermann D 2003 Pharmacokinetics in plasma and milk of benzylpenicillin following repeated intramuscular administration of mamyzin (penethamate hydriodide) in lactating cows. Journal of Veterinary Pharmacology and Therapeutics 26 (Suppl. 1) 100–101.

Fox LK 2009 Prevalence, incidence and risk factors of heifer mastitis. Veterinary Microbiology 134 82-88.

Gentilini E, Denamiel G, Betancor A, Rebuelto M, Rodriguez Fermepin M & De Torres RA 2002. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. Journal of Dairy Science 85 1913-1917.

Huijps K, De Vliegher S, Lam TJGM & Hogeveen H 2009 Cost estimation of heifer mastitis in early lactation by stochastic modelling. Veterinary Microbiology 134 121-127.

Kreiger M, Friton GM, Hofer J, Fuchs K & Winter P 2007 Effects of periparturient systemic treatment with penethamate hydriodide on udder health and milk yield of dairy heifers. Journal of Dairy Research 74 392-398.

Moroni P, Casula A, Spreafico G, Ruffo G & Bronzol V 2002. New strategy in management and control of Staphylococcus aureus intramammary infections in Italian dairy herd. Proceedings of the 41st NMC meeting Orlando, Florida, US 182-183.

Nickerson SC 2009 Control of heifer mastitis: antimicrobial treatment – An overview. Veterinary Microbiology 134 128-135.

Oliver SP & Mitchell BA 1983 Intramammary infections in primigravid heifers near parturition. Journal of Dairy Science 66 1180-1183.

Parker KI, Compton C, Anniss FM, Weir A, Heuer C & McDougall S 2007 Subclinical and clinical mastitis in heifers following he use of a teat sealant precalving. Journal of Dairy Science 90 207–218.

Piepers S, De Vliegher S, Opsomer G, de Kruif A & Barkema HW 2009 Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. Veterinary Microbiology 134 113-120.

Roberson JR, Fox LK, Hancock DD, Gay CC & Besser TE 1994 Coagulase-positive Staphylococcus intramammary infections in primiparous dairy cows. Journal of Dairy Science 77 958–969.

Salat O, Serieys F, Poutrel B, Durel L & Goby L (2008) Systemic treatment of subclinical mastitis in lactating cows with penethamate hydriodide. Journal of Dairy Science 91 632-640.

Serieys F, Raguet Y, Goby L, Schmidt H & Friton G 2005 Comparative efficacy of local and systemic antibiotic treatment in lactating cows with clinical mastitis. Journal of Dairy Science 88 93-99.

Tarbin J, Farrington WHH & Shearer G 1995 Determination of penicillins in animal tissues at trace residue concentrations. Part I. Determination of benzylpenicillin in milk by reversed-phase liquid chromatography with solid phase extraction and liquid chromatographic fractionation clean-up. Analytica Chimica Acta 318 95-101.

Trinidad P, Nickerson SC & Alley TK 1990 Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. Journal of Dairy Science 73 107-114.

Ziv G 1980 Practical pharmacokinetic aspects of mastitis therapy - 1: Parenteral treatment. Veterinary Clinics of North America Small Animal Practice 75 277-290.

Chapter 5.2

Systemic Prepartum Treatment of End-term Dairy Heifers with Penethamate Hydriodide: Effect on Udder Health, Milk Yield, and Culling until 120 days in milk

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Abstract

Prepartum intramammary treatment with antimicrobials of end-term dairy heifers has frequently been proposed as a practice to reduce the prevalence of intramammary infections (IMI) at calving. From a safety standpoint for both animal and administrator, systemic treatment is to be preferred. A clinical trial was conducted on heifers from 10 commercial, well-managed dairy farms with low prevalence of heifer mastitis. The aim was to assess both the short and long-term effects of a systemic prepartum therapy with penethamate hydriodide on udder health and milk production. Because it was hypothesized that some herds would benefit more from this treatment than others, specific herd-level information was collected prior to the start of the actual trial in order to screen for and/or explain potential herd-specific treatment effects. Further, the effect of treatment on antimicrobial susceptibility of Staphylococcal isolates was monitored. End-term heifers were either treated systemically (during three consecutive days) two weeks prior to expected calving date with penethamate hydriodide (n=76) or remained untreated (n=73). Systemic prepartum treatment of end-term heifers with penethamate hydriodide resulted in fewer IMI in early lactation. However, all 6 cases of CM in early lactation occurred in the treatment group [*Streptococcus uberis* (n = 1), *Corynebacterium bovis* (n = 1), *Staphylococcus aureus* (n = 1); one sample was contaminated and two samples remained culture negative]. No long-term treatment effects (4 to 120 DIM) on milk production, udder health or culling hazard during later lactation were detected although treated heifers belonging to herds classified as having low yielding heifers out-produced the control heifers. Moreover, penicillin susceptibility of Staphylococci isolated from milk samples of either treated or control heifers did not differ. Herds with a low prevalence of heifer mastitis are not likely to benefit from prepartum systemic antimicrobial treatment of the end-term heifers.

Introduction

Generally, bred heifers are assumed to have no issues with udder health and for that reason their mammary glands and secretions are often not checked until calving (Nickerson, 2009). However, a large proportion of dairy heifers calve with infected quarters (Fox, 2009). Studies have shown a wide variation in prevalence, from 29.0 to 74.6% (Oliver and Mitchell, 1983; Trinidad et al., 1990a) and from 12.3 to 55.0% (Roberson et al., 1994; Parker et al., 2007) of quarters being reported as culture positive before and at calving, respectively. A number of different pathogens have been isolated but studies have shown that infections are predominantly caused by Gram-positive bacteria, specifically CNS, *Staphylococcus aureus*, and environmental streptococci (Fox, 2009). Heifer mastitis can have a negative impact on future productive life (De Vliegher et al., 2004, 2005a,b), the impact depending on factors such as virulence of the pathogens involved and time of onset of the IMI during gestation (Piepers et al., 2009). The cost of subclinical heifer mastitis in early lactation alone on an average Dutch/Flemish dairy farm has been estimated to vary from €4 to €82 per heifer with an average of €31 (Huijps et al., 2009).

The use of prepartum antimicrobial treatment of end-term heifers in the control of heifer mastitis has been studied using short acting intramammary preparations, administered between 6 and 21d prior to calving. (Oliver et al., 1992, Oliver et al., 2004; Middleton et al., 2005; Borm et al., 2006; Roy et al., 2007) and long acting intramammary preparations, administered between 0 and 270 d prior to calving (Trinidad et al., 1990b; Owens et al., 1991, 1994, 2001; Sampimon et al., 2009). The majority of those studies showed positive effects on the short-term, as seen by higher cure rates of IMI detected before calving and/or a lower prevalence of IMI at calving in treated heifers compared with untreated controls (Nickerson, 2009). One could argue it makes more sense from an economical point of view to study the treatment effects on the longer term rather than on the short term. Trinidad et al. (1990b) studied milk production during the first two months of lactation and showed that *S. aureus*-infected heifers that had received prepartum dry cow therapy (penicillin and dihydrostreptomycin) produced an average of 2.5 kg more milk per day than *S. aureus*-infected herd mates that did not receive treatment. Oliver et al. (2004) showed that prepartum intramammary treatment using short acting preparations (penicillin-novobiocin and pirlimycin hydrochloride) was effective in reducing the percentage of infected heifers and quarters during the first 30 days in milk (DIM) whereas Sampimon et al. (2009) reported positive long-term effects of dry cow antimicrobial (cloxacillin) treatment 8 to 10 wk before the expected calving date on the incidence of CM, test-day SCC, and test-day milk yield (MY) in first lactation. The study of Oliver et al. (2004) is contrasted with the findings of Middleton et al. (2005) who

observed that intramammary treatment using short acting preparations (pirlimycin hydrochloride) did not necessarily reduce SCC or result in higher milk production during the first lactation although a higher overall cure rate at calving was noted.

Borm et al. (2006) concluded, based on the results of milk production, that prepartum treatment of end-term heifers with short acting intramammary preparations (cephapirin) was not uniformly efficacious across herds but potential herd-level factors explaining the findings were not studied further. Bryan and Taylor (2009) also reported a strong herd effect in their study demonstrating that systemic treatment with a single large dose of intramuscular penicillin within 12h after calving was successful in significantly reducing the incidence of CM in heifers within the first 7 DIM. Given these results, use of prepartum antimicrobial therapy in end-term heifers as a universal and economical viable strategy to increase milk production and improve udder health in heifers is not warranted. However, as some herds seem to benefit from treatment and others do not, it would be useful to understand why this is and what kind of factors are associated with that finding, specifically in the light of prudent and substantiated use of antimicrobials.

Systemic antimicrobial treatment of end-term heifers has a number of advantages over intramammary treatment: a lower risk of teat contamination, a higher convenience and safety to administer, and four quarters being treated with a single administration. Systemic use of penethamate hydriodide prior to calving was associated with penicillin G levels in mammary tissue and secretion substantially higher than the MIC90 of pathogens associated with heifer mastitis (Passchyn et al., 2010). However, the limited number of studies that have looked at systemic treatment showed either no effect (Parker et al., 2008 using tylosin) or a positive effect and were only conducted on problem herds (Kreiger et al., 2007 using penethamate hydriodide).

Antimicrobials are necessary for decreasing the prevalence and incidence of bacterial diseases in animals. Their use in veterinary medicine can have a positive effect on animal health, animal well-being and productivity, when used with sound clinical judgment combined with sound management practices (Johnston, 1998). Excessive and/or injudicious use of antimicrobials should, however, be avoided at all times. Taking the recent concerns related to the emergence of antimicrobial resistance in human and animal pathogens and the possible link with the use of antimicrobials in livestock into account, monitoring the development of antimicrobial resistance, even on the short term, in treatment trials obviously reflects good practice. Antimicrobial resistance of udder pathogens in Belgium (Annual Report 2011, Milk Control Centre Flanders, Lier, Belgium) is low and in line with other countries (Erskine, 2006).

Given all that information, a clinical trial was conducted on heifers from 10 commercial, well-managed dairy farms with low prevalence of heifer mastitis. The aim was to assess both the short and long-term effects of a systemic prepartum therapy with penethamate hydriodide 2 weeks before the expected date of calving on udder health and milk production. Because it was hypothesized that some herds would benefit more from this treatment than others, herd-level information was collected prior to the start of the actual trial in order to screen for and/or explain any herd-specific treatment effect. Further, the effect of treatment on susceptibility of Staphylococcal isolates from milk was monitored.

Materials and Methods

Herds, Heifers and Study Design

The study was conducted between September 2008 and June 2010 and included 229 heifers from 10 commercial, well-managed dairy herds, located in a radius of 20 km around Torhout, province of West Flanders, Belgium (Table 1). In total 80 heifers served as monitoring heifers, 76 were treated and 73 were untreated control heifers. Herd owners were approached by the first author and asked whether they were willing to participate. All herds had a good animal identification system, participated in the local dairy herd improvement (DHI) program (CRV, Oosterzele, Belgium) on a 4 to 6 weekly basis, and kept good records of treatments and diseases. Herds already treating end-term heifers with antimicrobials before calving were not approached.

Herd size ranged from 45 to 118 lactating cows with an average milk production of 9220 kg/cow per lactation, ranging from 8154 to 11,122 kg/cow. The geometric mean of the BMSCC) per herd in the 6-months period before the study started was 226,000 cells/mL ranging from 74,000 to 326,000 cells/mL. None of the herds reported a heifer mastitis problem, and none of the herds was considered to have such a problem when comparing the CM and SCC herd data using the thresholds published earlier [herd suffering from heifer mastitis if > 15% of heifers have CM around calving and/or if > 15% of all heifers have a first test-day SCC (measured between 10 and 35 DIM) > 150,000 cells/mL, De Vliegher et al., 2012]. All lactating cows and heifers were housed in free-stalls with cubicles and in all herds sawdust was used as bedding material.

Table 1. Overview of herd characteristics and management practices in the 10 commercial dairy herds participating in the study.

Herd Characteristics	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10
Average lactating cows (n)	118	54	58	51	45	64	86	83	65	62
305d-milk yield (kg)	9271	8971	11,122	8154	8780	8561	9856	8221	10,562	8709
Average herd SCC (x1000 cells/mL)	310	210	74	159	156	326	183	268	295	280
Udder health management										
Gloves worn during milking	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Post-milking teat dipping applied[1]	Yes[a]	Yes[b]	Yes[c]	Yes[c]	Yes[b]	Yes[b]	Yes[a]	Yes[b]	Yes[c]	Yes[b]
Dry-cow treatment										
Active component[2]	Cef	Clox	Cef	Clox	Cefq	Clox	Clox	Cef	Clox	Clox
Internal teat sealer applied	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
Calving pen										
Separate straw box	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Used for sick cows	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Cleaning of slatted floor	2x/day	2x/day	6x/day	2x/day	2x/day	2x/day	6x/day	2x/day	2x/day	1x/day
Heifers included (n)										
Treated	6	8	8	7	8	8	8	8	8	7
Control	7	8	8	6	6	8	8	6	8	8

[1] active component: [a] chlorhexidine, [b] iodine, [c] lactic acid & sodium chlorite, [2] cef = cefquinome, clox = cloxacilline.

Before the actual trial was started, herds were first monitored by sampling the first eight heifers per herd that calved (80 heifers in total) in order to construct a number of binomial herd-level predictor variables (Table 2 – see further). Milk samples were taken between 0 and 3 DIM for SCC measurement (composite milk) and bacteriological culture (quarter milk) as mentioned below. Monitoring of a herd ended after the 8th heifer had calved. Thereafter, the actual clinical trial started for this herd, comprising approximately an additional 16 heifers of which half were treated prior to calving and halve served as untreated controls. Heifers were alternately assigned by the author based on their expected calving date; every other heifer that was expected to calve was treated with penethamate. No placebo was used in the untreated control group. In total, 160 heifers, belonging to 10 herds (16 per herd), were aimed to be included in the actual treatment trial based on a sample size calculated using an expected difference in the proportion of infected animals in early lactation of 20% between the untreated and treated group (level of confidence 95%, power 80%; Winepiscope 2.0).

Treatment Regime

As mentioned, approximately eight end-term heifers per herd were treated systemically approximately 14 days (median 13.8d; interquartile range 10d to 16d) prior to the expected calving date, whereas another eight heifers per herd served as untreated controls. Heifers were only included if they were clinically healthy at the time of potential treatment, had not received any antimicrobial treatment in the last 4w prior to treatment and were excluded from the study if they calved within three days after treatment. Treatment consisted of daily IM injections of penethamate hydriodide (Mamyzin®/Stop M®, Boehringer Ingelheim GmbH, Ingelheim, Germany) for three consecutive days at a dose of 10 g/animal on the first day, followed by 5 g/animal on the second and third day (1µg of penethamate hydriodide provides 1 IU of penicillin G), in accordance with the approved commercial product label for France. Mamyzin® is registered in Belgium for treatment of clinical and subclinical mastitis in lactating cows, meaning that treatment of end-term heifers with Mamyzin® constitutes extra-label use. Extra-label use is allowed in Belgium under specific conditions and with implications for the withholding time of both meat and milk. All treatments were administered by the farmer.

Data and Sampling

All heifers were sampled by the first author once between 0 to 3 DIM (further referred as early lactation) for bacteriological culture (5 mL; duplicate quarter milk samples) and determination of milk SCC (30 mL; composite sample: samples of different quarters were combined using equal volumes), and were checked for signs of CM at that time. All milk samples were collected after disinfection of the teats and after the first streams of milk were discarded. Milk samples

were immediately stored at 4°C and then transported under cooled conditions to the laboratory (Milk Control Centre Flanders, Lier, Belgium).

Data on the occurrence of CM, culling and the reason for culling during the first 120 DIM were recorded by the farmer. Milk recording data (test-day milk production and composite SCC) of all heifers were collected with an interval of 4 to 6 weeks until 120 DIM.

Laboratory Analyses

Bacteriological Culture. Bacteriological culture was done as described previously (Piepers et al., 2007). Briefly, 0.01mL of milk was plated on a blood-esculin agar (Oxoid, Erembodegem, Belgium; 1 plate per cow) and on MacConkey's agar (Oxoid; 1 plate per cow). All plates were incubated aerobically for 36 ± 12 h at 37 ± 1°C. A quarter was considered culture-positive when growth of ≥ 1 colony was detected. Samples yielding 3 or more different bacterial species were considered to be contaminated. Bacteria were identified by colony morphology and Gram-staining. For Gram-positive cocci, catalase tests were used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Colony morphology, hemolysis patterns, and DNase testing were used to distinguish *Staph. aureus* from CNS. Streptococci were subdivided into esculin-positive streptococci (*Streptococcus uberis*) and esculin-negative streptococci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*). Differentiation between *Streptococcus uberis* and other streptococci was done using bile aesculine agar and NaCl 6.5%. The Christie, Atkins, Munch-Petersen (CAMP) test was used to differentiate *Strep. agalactiae* from *Strep. dysgalactiae*. Coliforms including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. were differentiated from each other and from other Gram-negative bacteria based on the appearance on MacConkey's agar, KOH-testing, Triple Sugar Iron reactions, indol production and motility. *Staphylococcus aureus*, esculin-positive streptococci, *Strep. agalactiae*, *Strep. dysgalactiae* and coliforms were considered as major pathogens. Coagulase-negative staphylococci and *Corynebacterium bovis* were considered as minor pathogens.

Penicillin Resistance. All Staphylococci were tested by the Etest method (AB BIODISK, Solna, Sweden), a stable-gradient agar diffusion technique that produces quantitative MIC results over a 15 $\log^2$ dilution range (Brown and Brown, 1991). Isolates were defined as "susceptible" to penicillin G when the MIC was ≤ 0.125 mg/L and as "resistant" to penicillin G when the MIC was > 0.125 mg/L, respectively (EUCAST, 2011).

Somatic Cell Count. Milk SCC was quantified by electronic counting using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) at the Milk Control Centre Flanders (Lier, Belgium).

Table 2. Binomial herd-level predictor variables constructed using data from the monitoring heifers prior to the start of the actual clinical trial and used to screen for potential herd-dependent treatment effects.

Variable	Recording method	Description	Break down categories	Average per category
CNS heifers	Culture	% CNS infected quarters of monitoring heifers	High (≥ 18.5%) *versus* Low (< 18.5%)[1]	20.6% 7.3%
Majors heifers	Culture	Presence of major pathogens in monitoring heifers	Presence *versus* Absence	8.7% 0%
305d milk yield	DHI-records	Arithmic average 305d milk yield of the lactating herd	High (≥ 8.875kg) *versus* Low (< 8.875kg)[1]	9.956 kg 8.485 kg
Milk yield	DHI-records	Milk yield at first test-day of the lactating heifers	High (≥ 25.8kg) *versus* Low (< 25.8kg)[1]	27.8 kg 24.4 kg
Average heifer SCC	DHI-records	Arithmic average test-day SCC of the lactating heifers	High (> 150.000 cells/mL) *versus* Low (≤ 150.000 cells/mL)[1]	281.000 cells/mL 99.000 cells/mL
Average herd SCC	DHI-records	Arithmic average test-day SCC of the lactating herd	High (> 200.000 cells/mL) *versus* Low (≤ 200.000 cells/mL)[1]	281.000 cells/mL 143.000 cells/mL
Presence of penicillin resistance	E-test	Susceptibility to penicillin of staphylococcal isolates	Presence *versus* Absence	24% 0%
Hygiene herd	Visual	Percentage of unclean monitoring heifers	Unclean (≥ 37.5%) *versus* Clean (< 37.5%)[1]	65.6% 27.1%

[1]Categorization based on median value.

Antimicrobial Residues. Before delivering milk (2-4 DIM), farmers tested all treated heifers as required by the Dairy Quality Assurance scheme with a Delvo-test (Angelidis et al., 1999). No residues were detected in any of the samples.

Definition of Subclinical and Clinical Mastitis

A quarter was considered subclinically infected in early lactation (0 - 3 DIM) when, in the absence of clinical symptoms, the same udder pathogen was isolated from both duplicate milk samples (Borm et al., 2006). A quarter was considered as non-infected when no pathogens were isolated from both duplicate milk samples. When only in one sample from both duplicate milk samples an udder pathogen was isolated or when one or both milk samples were contaminated the data were considered missing.

Clinical mastitis was recorded by the first author and/or the farmer and was defined as the presence of visual signs such as clots in the milk, with or without redness, swelling of the udder quarter, or systemic signs. The interval between cases of CM in the same quarter had to be ≥ 14d to be included in the analysis as a new CM case (Barkema et al., 1998). All CM were treated by the farmer according to the farms' treatment protocol.

Herd-Level Predictor Variables

As aforementioned, a number of binomial herd-level predictor variables were constructed using the data gathered from the monitoring heifers (n = 80) prior to the start of the actual clinical trial (Table 2). These variables were used to screen for potential herd-dependent treatment effects. The distribution of these herd-level predictor variables over the different herds is visualized in Table 3. Hygiene scores ranging from 1 (clean) to 5 (dirty) were assigned for four body areas: tail head, thigh, udder, and hind limbs (Hughes, 2001). Heifers with an average cleanliness score ≤ 2.0 (median value of all herd averages) were considered as "clean" and heifers with a cleanliness score > 2.0 were considered as "unclean".

Statistical Analyses

Prior to statistical analysis, observations were explored and checked for unlikely values. No data were excluded for this reason. Milk SCC was transformed to the natural logarithmic scale (LnSCC) to normalize the data. Significance level was set at $P < 0.05$.

Table 3. Distribution of the binomial herd-level predictor variables[1] that were used to screen for potential herd-dependent treatment effects over the participating herds.

	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	Herd 7	Herd 8	Herd 9	Herd 10
CNS heifers	Low	High	Low	Low	High	High	High	High	Low	High
Majors heifers	Presence	Presence	Absence	Absence	Presence	Presence	Presence	Presence	Presence	Presence
305d Milk yield	High	High	High	Low	Low	Low	High	Low	High	Low
Milk yield heifers	Low	High	High	Low	High	Low	High	Low	High	Low
Average heifer SCC	High	Low	Low	Low	Low	Low	High	Low	High	High
Average herd SCC	High	Low	Low	High	Low	High	High	High	High	High
Presence of penicillin resistance	Presence	Presence	Presence	Absence	Presence	Presence	Absence	Absence	Presence	Presence
Hygiene herd	Unclean	Unclean	Unclean	Clean	Clean	Unclean	Clean	Unclean	Clean	Unclean

[1]See Table 2 for definitions.

In general, 8 different outcome variables were used. Outcomes in early lactation (0-3 DIM) were: likelihood of IMI due to any pathogen, due to major pathogens only or due to CNS only, and prevalence of CM. Outcomes in late lactation (4-120 DIM) were: LnSCC at test-day, MY at test-day, incidence of CM and culling. For 5 of the 8 different outcome variables (likelihood of IMI in early lactation due to any pathogen, major pathogens only, or CNS only; LnSCC and MY at test-day) separate models were fitted using a common approach including the following predictor variables: treatment (yes versus no), one of the 8 binomial herd-level predictor variables (Table 2), and the interaction between treatment and the included herd-level predictor variable. Each of the 8 binomial herd-level predictor variables (Table 2) was added to each model, then again removed and replaced by the next one which eventually resulted in 8 separate models per outcome variable. The models were reduced if the interaction term was not significant ($P > 0.05$). The association between treatment (yes versus no) and the likelihood of IMI at the quarter level with either any pathogen, major pathogens only or CNS only in early lactation as the different outcome variables was analyzed by means of logistic mixed regression models using 1st order marginal quasi-likelihood algorithms (MLwiN 2.16 - Centre for Multilevel Modeling, Bristol, UK). In each model, heifer and herd were included as random effects to correct for clustering of quarters within heifers and heifers within herds. The association between treatment (yes versus no) and MY and SCC at test-day during the first 120 DIM, respectively, were analyzed using mixed models (SAS version 9.1.3 - SAS Institute Inc., Cary, NC, USA) with test-day MY (kg) and LnSCC as outcome variables. Herd and heifer were included as random effects to correct for clustering of heifers within herds and repeated measurements within heifers. An autoregressive (1) covariance structure was included to model the repeated measurements within heifers. Because of the repeated measurements, DIM (continuous) at test-day and the quadratic effect of DIM were included in the models besides the aforementioned predictor variables.

The association between treatment (yes versus no) and prevalence of CM in early lactation (0 - 3 DIM), incidence of CM in later lactation (4 - 120 DIM), and culling in later lactation, respectively, were examined using contingency tables and $\chi 2$ analysis. Correction for clustering of heifers within herds was not feasible because of limited number of events. For the same reason, herd predictors and their interaction with treatment were not tested.

To compare antimicrobial susceptibility of staphylococcal isolates cultured from milk collected in early lactation (subclinical samples) from treated and control heifers, survival curves of both groups was plotted using a Kaplan-Meier survival analysis (SPSS version 19.0., SPSS Inc., Chicago, IL), with the MIC values defined as the time-to-event (Sampimon et al., 2011). When isolates were killed below the lowest test concentration or were still growing at the highest test

Table 4. Quarter-level prevalence of IMI in early lactation (0-3 DIM) and distribution of pathogens from the heifers sampled prior to the onset of the clinical trial (Monitoring) as well as from the heifers included in the clinical trial, either systemically treated prepartum with penethamate hydriodide (Treated) or left untreated (Control).

	Monitoring		Treated		Control		
Infection status (quarter level)	n	%[1]	n	%[1]	n	%[1]	*P-value*[2]
Non-infected	168	52.5	187	61.5	160	54.8	
Subclinically infected with any pathogen	95	29.7	54	17.9	74	25.4	0.023
Subclinically infected with major pathogens	27	8.4	14	4.6	20	6.8	0.173
Staphylococcus aureus	6		1		5		
Streptococcus uberis	9		6		4		
Streptococcus dysgalactiae	4		1		2		
Other streptococci			4		7		
Escherichia coli	8		2		2		
Subclinically infected with minor pathogens	68	21.3	42	13.3	57	18.6	
Coagulase-negative staphylococci	65	20.3	40	13.1	54	18.5	0.053
Other (*Bacillus sp., Corynebacterium bovis*)	3	<1	2	<1	3	<1	
Contaminated	23	7.2	19	6.2	18	6.2	
Missing values	34	10.6	44	14.4	40	13.6	
Total	320		304		292		

[1]Percentage of total quarters sampled, [2]*P*-value from logistic mixed regression models with herd and heifer as random effect comparing odds of quarters subclinically infected with any pathogen, major pathogens only, or coagulase-negative staphylococci only, respectively versus non-infected quarters from treated and control heifers.

concentration, observations were coded as left of right censored, respectively. The Logrank test was used to test for significant differences in survival between the two groups of isolates ($P < 0.05$).

Results

Descriptive Results

In total, 149 heifers (596 quarters) of which 76 treated heifers (304 quarters) and 73 control heifers (292 quarters) were included in the clinical trial. The target of 80 heifers in each group was not met since herd sizes were sometimes not sufficient to reach the required number of heifers within the specified time frame.

Overall, in early lactation 347 quarters were non-infected, 34 had an IMI with a major pathogen, and 94 with CNS (Table 4). Samples from 37 quarters were contaminated and for 84 quarters assignment of an IMI status was impossible (all data encoded as missing values). In total, 128 quarters had an IMI with a major pathogen or with CNS (Table 4). In addition, six heifers suffered from a CM at calving (4%). All (n = 12) of the *Staphylococcus aureus* isolates and 73% (n = 143) of the CNS isolates originating from milk samples taken in early lactation during the treatment trial were susceptible to penicillin.

The overall average SCC at first test-day was 290,221 cells/mL (SD = 725,521) and decreased to 122,000 cells/mL (SD = 338,819) at the fourth test-day. Average milk production started at 27.0 kg/day (SD = 5.9) at first test-day, then rose to 30.2 kg/day (SD = 5.6), to finally end at 27.7 kg (SD = 5.3) at the fourth test-day. Thirteen heifers developed a case of CM during their first 120 DIM, whereas seven heifers were culled during that period of which one because of mastitis issues.

Treatment Effects

In all herds, quarters from penethamate-treated heifers were significantly less likely to have an IMI in early lactation with any pathogen ($P = 0.023$). A trend towards fewer IMI with CNS was also noticed ($P = 0.053$) (Table 4). The likelihood of IMI with major pathogens did not differ between treated and non-treated heifers ($P = 0.17$). None of the herd-level predictors or interactions with treatment was significant, indicating the treatment effects were not modified by any of the studied herd-level predictor variables.

All 6 cases of CM in early lactation occurred in the treatment group. *Strep. uberis* (n = 1), *Corynebacterium bovis* (n = 1), *S. aureus* (n = 1) were isolated; one sample was contaminated and two samples remained culture negative.

Seventy one percent (n = 60) and 74% (n = 83) of all *Staphylococcus* isolates recovered from milk samples from the control and treated heifers in early lactation, respectively, were susceptible to penicillin (P > 0.05) (Figure 3).

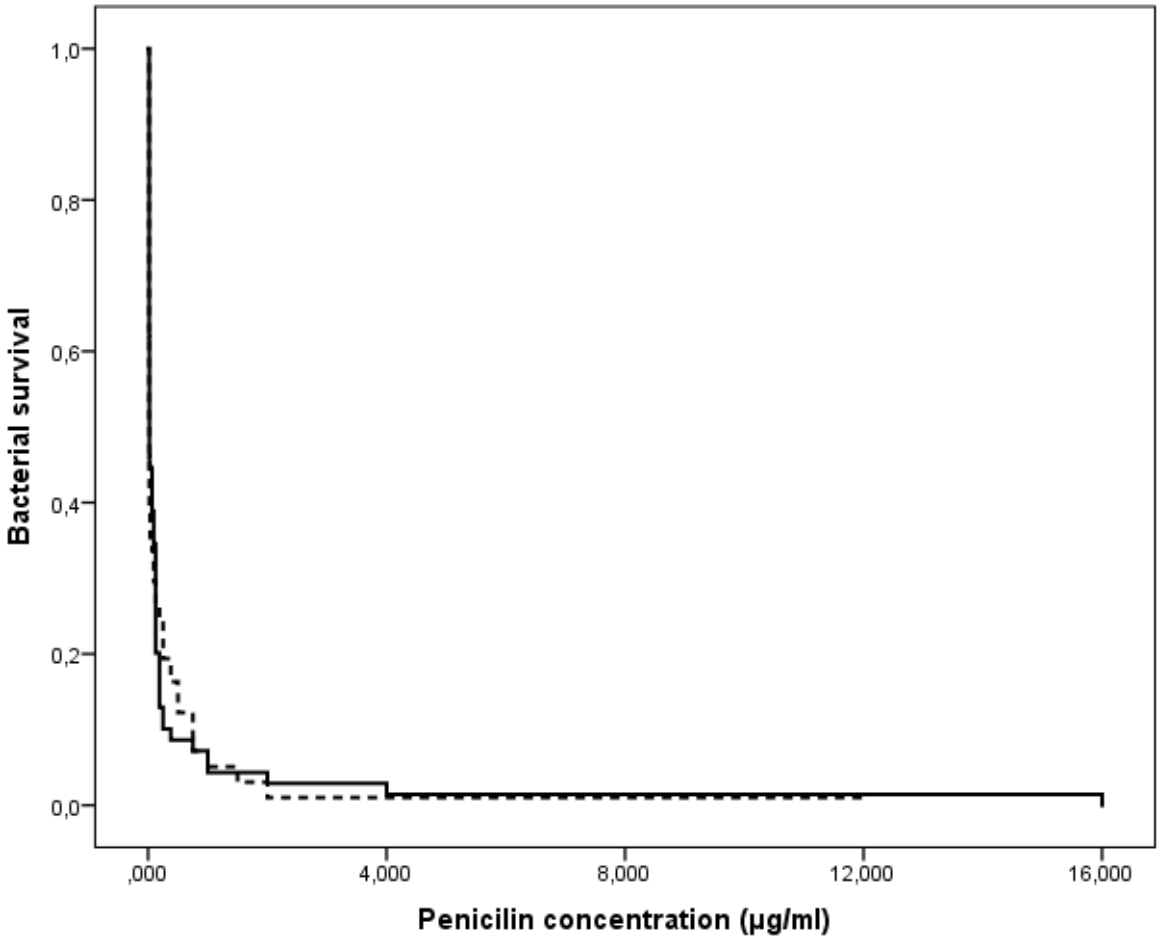

Figure 3. Kaplan-Meier graph of the proportion of Staphylococcal spp. isolates surviving with increasing concentrations of penicillin, cultured from samples taken at calving from control (---) and treated (—) heifers (*P* = 0.81).

Treatment did not influence LnSCC at test-day during the first 120 DIM (P = 0.85) (Figure 1, Table 5). None of the herd-level predictor variables or interaction terms was significant and therefore omitted from the final models.

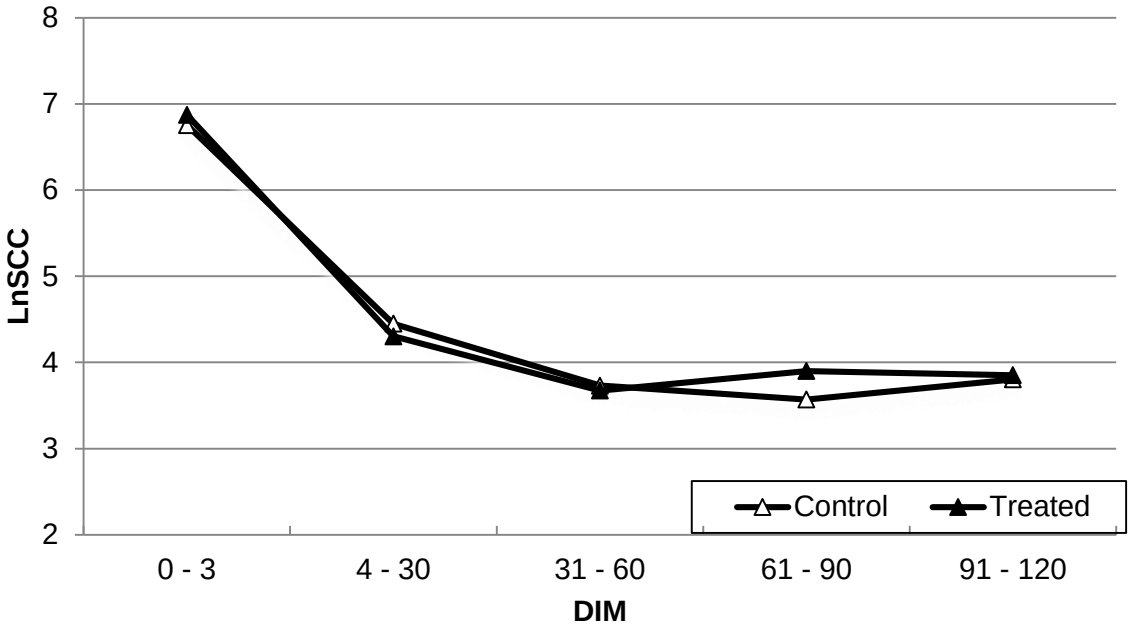

Figure 1. Average LnSCC in heifers systemically treated prepartum with penethamate hydriodide and control heifers during 120 DIM (*P* = 0.85).

Treatment did not influence test-day MY during the first 120 DIM (*P* = 0.87, Table 5). None of the herd-level predictor variables was significant, although one interaction term was (MY heifers x Treatment; *P* = 0.02). Penethamate-treated heifers from herds classified as having low yielding heifers out-produced untreated animals (+2.2 kg milk/day, *P* = 0.07) whereas in herds classified as having high yielding heifers, milk production was 1.5 kg milk per day lower in treated heifers compared with the controls (*P* = 0.14) (Figure 2). Also, treatment tended to be associated with a higher test-day MY in farms with a high average herd SCC than on farms with a low average herd SCC (Average Herd SCC x Treatment; P = 0.08).

Five (6.8%) control heifers developed a case of CM between 4 to 120 DIM, whereas 8 (10.5%) treated heifers suffered from a case of CM (uncorrected χ^2= 0.21; *P* = 0.63) (Table 5).

In the control group, only one heifer was culled (because of mastitis), whereas in the treatment group, 6 heifers were culled, but none of them because of mastitis issues: three heifers were culled because of trauma, two were culled because of non-functional quarters and one because of claw disorders (Table 5).

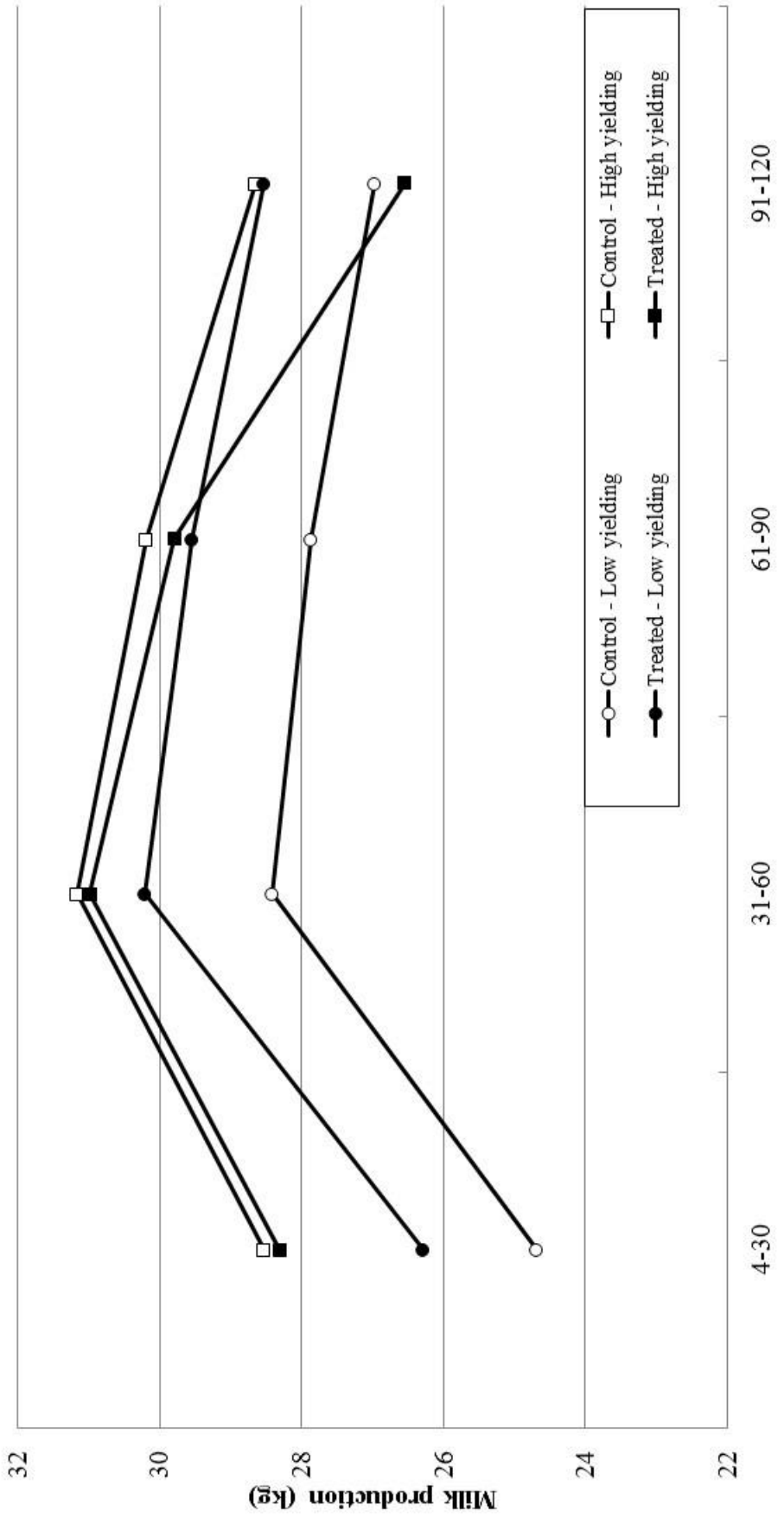

Figure 2. Test-day milk production (kg/d) in heifers systemically treated prepartum with penethamate hydriodide (treated) and untreated heifers (control) during 120 DIM in herd categorized as having low yielding heifers versus high yielding heifers (interaction term ; P =0.02).

Table 5. Overview of data on udder health, milk production and culling hazard of the dairy heifers included in the clinical trial either systemically treated prepartum with penethamate hydriodide (Treated) or untreated (Control) in later lactation (4 - 120 DIM).

	Average test-day LnSCC[1] (x1000 cells/mL)		Average test-day milk yield[1] (kg)		CM (n)		Culling for all reasons[2] (n)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Herd 1	5.00	4.71	31.0	29.1	0	0	0	1[2]
Herd 2	4.99	4.62	26.5	29.6	2	3	2	0
Herd 3	3.70	4.10	33.7	33.7	0	0	0	0
Herd 4	5.10	5.02	27.4	24.1	0	0	1	0
Herd 5	4.50	4.35	27.5	29.6	0	0	0	0
Herd 6	4.41	4.28	28.1	26.8	1	1	0	0
Herd 7	4.59	4.15	30.2	30.9	1	0	0	0
Herd 8	4.77	4.69	29.8	29.2	3	1	1	0
Herd 9	3.94	4.67	25.5	28.3	0	0	2	0
Herd 10	4.61	4.75	31.1	26.8	1	0	0	0
Total	4.56	4.57[3]	31.0	29.2[4]	8	5[5]	6	1[6]

[1]Least square means based on models including herd and herd x treatment interaction as fixed effects, [2]Denotes that the heifer was culled because of mastitis (indicated culling reason), [3]Differences between treated and control heifers was not significant (P = 0.85), [4]Differences between treated and control heifers was not significant (P = 0.87, [5]Differences between treated and control heifers was not significant (P = 0.63), [6]The model looking whether numbers were statistically different did not converge as only one heifer was culled due to mastitis.

Discussion

Prevalence of IMI with any pathogen was 25.3% at the quarter level in the control group, which was within the range of 12 to over 57% stated by De Vliegher et al. (2012) reviewing a number of different studies. The majority of IMI were with CNS, substantiating the finding of most other studies, whereas IMI with major pathogens were in the minority. The sole fact that CNS infections are abundantly present in milk samples from heifers at calving should not be interpreted as if a heifer mastitis problem exists as their impact on future performances is limited or even absent (Compton et al., 2007; Piepers et al., 2010; Piepers et al., 2013).

From a practical standpoint, the administration of antimicrobials to end-term heifers by systemic route is to be preferred over intramammary infusion (Nickerson, 2009). However, it has been questioned whether subcutaneous or intramuscular injection of drugs can actually cure IMI in bred heifers as insufficient concentrations of the antimicrobial might reach the mammary gland (Nickerson, 2009). This is most probably the first field trial that demonstrates the mammary gland has been reached by penethamate hydriodide as the systemic prepartum treatment was associated with significantly less IMI with both CNS and any pathogen in early lactation. This substantiates our previous work showing that substantially higher levels of penicillin G than the MIC90 of pathogens associated with heifer mastitis were reached in udder tissue and mammary secretions from heifers systemically treated with penethamate hydriodide prior to calving (Passchyn et al., 2010). Still, our findings were in contrast with those reported by Parker et al. (2008) who found that systemic treatment of end-term heifers with tylosin 27 days prior to calving, did not reduce the prevalence of IMI postcalving. All other studies on prepartum use of antimicrobials in the prevention of heifer mastitis that showed a significant decrease in IMI after treatment were performed using either short acting (Oliver et al., 1992; Oliver et al., 2004; Middleton et al., 2005; Borm et al., 2006; Roy et al., 2007) or long acting intramammary preparations (Trinidad et al., 1990b; Owens et al.,1991; Owens et al., 1994; Sampimon et al., 2009).

Surprisingly, all 6 cases of CM in early lactation occurred in the treatment group. Several of those CM might have been caused by gram-negative bacteria. In those cases, no effect of a penethamate treatment would have been anticipated. Iatrogenic infections by treatment as observed by Deluyker et al. (2005) could be excluded as no local treatment was applied in this study. Because not all CM cases are a function of pre-partum IMI and no antimicrobial residues were detected in the milk which means that penethamate hydriodide was not active at therapeutic levels in the mammary gland at calving, the higher incidence of CM in the treatment group could also be the result of chance. However, one could speculate that

treatment with penethamate has disturbed the micro-flora in the udder (Oikonomou et al., 2012), making the quarters of treated animals more susceptible to environmental bacteria although this needs further study.

From an economical point of view, positive long-term treatment effects are needed to support prepartum treatment of end-term heifers. Apart from one specific effect, no overall positive long-term effects (4 to 120 DIM) were detected during our trial. The latter observation is in contrast to the results reported by several other research groups (Trinidad et al.,1990b; Owens et al.,1991; Owens et al., 1994; Oliver et al.,2003; Sampimon et al. 2009) applying long acting intramammary preparations, but agrees with the findings of Borm et al. (2006) using a short acting intramammary preparation. As intramammary treatment with short acting preparations and systemic injection(s) have only a short period of curative or preventative action, those findings are probably not that surprising; long acting intramammary preparations, on the other hand, are expected to result in high drug concentration in the mammary tissue during several weeks.

Also, the herds included in the studies that reported a positive effect of prepartum treatment on MY had a high prevalence of major pathogen IMI and had a history of heifer mastitis problems (Kreiger et al., 2007; Bryan and Taylor, 2009; Sampimon et al., 2009). Some trials were conducted on research farms (Oliver et al., 1992; Owens et al., 1994; Oliver et al., 2004) or on farms having (suggested) heifer mastitis problems (Kreiger et al., 2007; Bryan and Taylor, 2009; Sampimon et al., 2009). The fact that none of the herds included in our study actually suffered from a heifer mastitis issue most probably explains why no overall beneficial long-term treatment effects (4 to 120 DIM) were detected. Our findings also reinforce that the sole fact of a high proportion of heifers/quarters infected with CNS in early lactation should not be a worry. In that respect, analysis of the data from the non-treated heifers only in recent study substantiated (Piepers et al., accepted) a previously described positive association between CNS IMI and milk yield (Piepers et al., 2010).

Given the conclusions of previous studies (Borm et al.,2006; Bryan and Taylor, 2009), our trial was specifically designed in anticipation of potential herd-specific treatment effects by including a monitoring period in the study prior to the onset of the actual treatment trial. This novel approach allowed us to detect that treated heifers belonging to herds classified as having low yielding heifers out-produced the control heifers. We hypothesized that the positive effect of prepartum treatment of heifers 2 weeks before calving could depend on the management. It is indeed well established that treatment of subclinical mastitis is more effective in well-managed than in poorly-managed herds where cured cows or heifers are more likely to become re-infected (Barlow et al., 2009). Assuming that the milk production of heifers

in our study reflects management, the latter hypothesis could not be substantiated and our data even suggest the opposite; all the more so since treatment tended to be more beneficial on high SCC herds than on low SCC herds. Still, none of the other herd-level predictors specifically reflecting the farmers' style of heifer-management such as SCC of fresh heifers and hygiene of the heifers modified the treatment effect in a similar way, leaving the exact explanation of the finding unknown. Based on the herd predictor that encoded for the presence of major pathogens, the hypothesis put forward by Barlow et al. (2009) could not be substantiated either. Still, caution should be taken when jumping to conclusions as the prevalence of IMI with major pathogens was small in our study. Although the herd-level predictors were selected based on their biological relevance and practical feasibility, studying 8 of them when only including 10 herds might have resulted in finding a significant association by coincidence.

Given the concerns that antimicrobial overuse in food animal production has the potential to increase antimicrobial resistance in human pathogens, alternative strategies to the widespread antimicrobial use are needed (FAO/WHO/OIE, 2007; Silbergeld et al., 2008). In that respect, prepartum treatment of end-term heifers, at a time heifers are not producing milk, would only be defensible if this would prevent additional treatments during lactation, at a time milk is being produced as a marketable product and when this would reduce animal welfare issues caused by CM. Also, treatment of end-term heifers consists of extra-label use in many countries which obviously has implications for the withholding time of both meat and milk. One could hypothesize that selection towards resistant commensals (such as CNS) is more likely to occur when systemic treatment is being used. In our study, treatment did not increase the likelihood of penicillin resistance in staphylococci (of which a majority was CNS) isolated in milk samples at calving. However, selection and spread of resistance takes time, making it difficult to conclude on this issue. Anyway, without over interpreting the data and in the light of antimicrobial resistance, the use of short-acting antimicrobials (intramammary and parenteral use) with a narrow spectrum should be preferred in those herds that suffer from a true heifer mastitis problem and that are hence likely to profit from treatment.

Conclusions

Systemic therapy of end-term heifers with penethamate hydriodide 2 weeks before calving resulted in less IMI at the quarter level in early lactation on the one hand but in more cases of CM in the first days after calving (0-3 DIM) on the other hand. Overall long-term effects (4 to 120 DIM) of penethamate treatment on milk production, udder health or culling hazard during

later lactation were not detected. Still, using the data from the monitoring period, it was shown that penethamate-treated heifers from herds classified as having low yielding heifers out-produced control heifers. Treatment of end-term heifers was not associated with the development of penicillin resistance in staphylococci isolated from milk samples on the short-term. Prepartum systemic antimicrobial treatment cannot be warranted in herds with low prevalence of heifer mastitis. A similar study conducted on commercial farms with a high prevalence of heifer mastitis is required to elaborate further the outcomes of this trial.

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References

Angelidis A.S, T.B. Farver , and J.S. Cullor. 1999. Evaluation of the Delvo-X-Press Assay for Detecting Antibiotic Residues in Milk Samples from Individual Cows. J. Food. Prot. 62:1183-1190.

Barkema, H. W., Y. H. Schukken, T. J. G. M. 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. 81:411-419.

Barlow, J. W., L. J. White, R. N. Zadoks, and Y. H. Schukken. 2009. A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds. Prev. Vet. Med. 90:31-42.

Borm, A. A., L. K. Fox, K. E. Leslie, J. S. Hogan, S. M. Andrew, S. P. Oliver, Y. H. Schukken, D. D. Hancock, C. T. Gaskins, W. E. Owens, and C. Norman. 2006. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. J. Dairy Sci. 89:2090-2098.

Brown, D.F.J., and L. Brown.1991. Evaluation of the Etest: a novel method of quantifying antimicrobial activity. J. Antimicrob. Chemother. 27:185–190.

Bryan, M. and K. Taylor. 2009. Periparturient use of parenteral micronised procaine penicillin to reduce the risk of clinical mastitis in heifers after calving. Vet. Microbiol. 134:143-149.

Compton, C. W. R., C. Heuer, K. Parker, and S. McDougall. 2007. Epidemiology of mastitis in pasture-grazed peripartum dairy heifers and its effects on productivity. J. Dairy Sci. 90:4157-4170.

Deluyker, H.A, S.N. Van Oye, and J.F. Boucher. 2005. Factors affecting cure and somatic cell count after pirlimycin treatment of subclinical mastitis in lactating cows. J. Dairy Sci 88:604-614.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Impact in dairy heifers of early lactation somatic cell count on somatic cell counts during the first lactation. J. Dairy Sci. 87:3672-3682.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2005a. Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. J. Dairy Sci. 88:938-947.

De Vliegher, S., H. W. Barkema, G. Opsomer, A. de Kruif, and L. Duchateau. 2005b. Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. J. Dairy Sci. 88:569-576.

De Vliegher, S., L.K. Fox, S. Piepers, S. McDougall, and H.W. Barkema. 2012. Invited review: mastitis in dairy heifers. J. Dairy Sci. 95 :1025–1040.

Erskine. 2006. Overview of literature on antimicrobial resistance of mastitis pathogens. Pages 3-9 in Proc. 45th Annual Meeting National Mastitis Council.

EUCAST. 2011. Clinical Breakpoint Table. http://www.eucast.org/antimicrobial_susceptibility_testing/previous_versions_of_tables/

Fox, L. K. 2009. Prevalence, incidence and risk factors of heifer mastitis. Vet. Microbiol. 134:82-88.

Hughes, J. 2001. A system for assessing cow cleanliness. In Pract. 23:517-524.

Huijps, K., S. De Vliegher, T. Lam, and H. Hogeveen. 2009. Cost estimation of heifer mastitis in early lactation by stochastic modeling. Vet. Microbiol. 134:121-127.

Johnston, A. M. 1998. Use of antimicrobial drugs in veterinary practice. BMJ 317:665–667.

Kreiger, M., G. Friton, J. Hofer, K. Fuchs, and P. Winter. 2007. Effects of periparturient systemic treatment with penethamate hydriodide on udder health and milk yield of dairy heifers. J. Dairy Res. 74:392-398.

Middleton, J. R., L. L. Timms, R. Bader, J. Lakritz, C. D. Luby, and B. J. Steevens. 2005. Effect of prepartum intramammary treatment with pirlimycin hydrochloride on prevalence of early first-lactation mastitis in dairy heifers. J. Am. Vet. Med. Assoc. 27:1969-1974.

Nickerson, S. C. 2009. Control of heifer mastitis: Antimicrobial treatment - an overview. Vet. Microbiol. 134:128-135.

Oikonomou, G., V.S. Machado, C. Santisteban, Y.H. Schukken, and R.C. Bicalho. 2012. Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomic 16s rDNA. PLoS One. 7:e47671.

Oliver, S. P., and B. A. Mitchell. 1983. Intramammary infections in primigravid heifers near parturition. J. Dairy Sci. 66:1180-1183.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, and H. H. Dowlen. 1992. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. J. Dairy Sci. 75:406-414.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Jaenicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. J. Dairy Sci. 86:1187-1193.

Oliver, S. P., B. E. Gillespie, S. J. Ivey, M. J. Lewis, D. L. Johnson, K. C. Lamar, H. Moorehead, H. H. Dowlen, S. T. Chester, and J.W. Hallberg. 2004. Influence of prepartum pirlimycin hydrocholoride or penicillin-novobiocin therapy in mastitis in heifers during early lactation. J. Dairy Sci. 87:1727-1731.

Owens, W. E., S. C. Nickerson, P. J. Washburn, and C. H. Ray. 1991. Efficacy of a cephapirin dry cow product for treatment of experimentally induced Staphylococcus aureus mastitis in heifers. J. Dairy Sci. 74:3376-3382.

Owens, W. E., S. C. Nickerson, P. J. Washburn, and C. H. Ray. 1994. Prepartum antibiotic-therapy with a Cephapirin dry-cow product against naturally-occurring intramammary infections in heifers. J. Vet. Med. Ser. B. 41:90-100

Owens, W. E., S. C. Nickerson, R. L. Boddie, G. M. Tomita, and C. H. Ray. 2001. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. J. Dairy Sci. 84:814-817.

Parker, K. I., C.W. Compton, F. M. Anniss, A. M. Weir, and S. McDougal. 2007. Management of dairy heifers and its relationships with the incidence of clinical mastitis. N. Z. Vet. J. 55:208-216.

Parker, K. I., C. W. Compton, F. M. Anniss, C. Heuer, and S. McDougall. 2008. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic, or both, precalving. J. Dairy Sci. 91:169-181.

Passchyn, P., S. Piepers, E. Schmitt, C. Guidarini, and S. De Vliegher. 2010. Concentration of penicillin G in mammary tissue and secretion of end-term dairy heifers following systemic prepartum administration of penethamate hydriodide. J. Dairy Res. 77:33-36.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H. W. Barkema, and S. De Vliegher. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res. 74:478-483.

Piepers, S., S. De Vliegher, A. de Kruif, G. Opsomer, and H. W. Barkema. 2009. Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. Vet. Microbiol. 134:113-120.

Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than non-infected heifers. J. Dairy Sci. 93: 2014-2024.

Piepers S., Y.H. Schukken, P. Passchyn, and S. De Vliegher. 2013. The effect of intramammary infection with coagulase-negative staphylococci in early lactating heifers on milk yield throughout first lactation revisited. Journal of Dairy Science, Vol. 96, Issue 8, p5095–5105.

Roberson, J. R., L. K. Fox, D. D. Hancock, J. M. Gay, and T. E. Besser. 1994. Coagulase-positive Staphylococcus intramammary infections in primiparous dairy cows. J. Dairy Sci. 77:958-969.

Roy, J. P., D. Du Tremblay, L. Descôteaux, S. Messier, D. Scholl, and E. Bouchard. 2007. Effect of precalving intramammary treatment with pirlimycin in nulliparous Holstein heifers. Can. J. Vet. Res 71:283-291.

Sampimon, O. C., S. De Vliegher, H. W. Barkema, J. Sol, and T. J. G. M. Lam. 2009. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. J. Dairy Sci. 92:4395-4403.

Sampimon, O. C., T. J. G. M. Lam, D. J. Mevius, Y. H. Schukken, and R.N. Zadoks. 2011. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. Vet. Microbiol. 150:173-179.

Silbergeld, E. K., J. Graham, and L. B. Price. 2008. Industrial food animal production, antimicrobial resistance, and human health. Annu. Rev. Public Health. 29:151-169.

Trinidad, P., S. C. Nickerson, and T. K. Alley. 1990a. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. J. Dairy Sci. 73:107–114.

Trinidad, P., S. C. Nickerson, T. K. Alley, and R. W. Adkinson. 1990b. Efficacy of intramammary treatment in unbred and primigravid dairy heifers. J. Dairy Sci. 197:465-470.

Chapter 5.3

Pathogen Group-specific Risk Factors for Intramammary Infection in Treated and Untreated Dairy Heifers participating in a Prepartum Antimicrobial Treatment Trial

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Abstract

Heifer mastitis is a well-known problem with a number of pathogens being involved. A number of generic risk factors associated with the likelihood of IMI in fresh dairy heifers have been identified before. Yet there is a need to identify pathogen group specific factors as the impact of (groups of) pathogens on udder health and milk yield is different. The aim of the present study was to identify pathogen group specific risk factors for IMI in heifers participating in a prepartum antimicrobial treatment trial, allowing us to test the hypothesis that different factors are of importance between treated and untreated control heifers as well. Data from a clinical trial in which end-term heifers were treated systemically (over 3 consecutive days) 2 wk before calving with penethamate hydriodide (n = 76) or remained untreated (n = 73), were available. A number of potential risk factors at the herd, heifer and quarter level were recorded in the first 3 DIM.

Quarters from untreated heifers supplemented with ≥ 4 mg selenium/day prepartum were significantly less likely to be infected with CNS whereas quarters were more likely to be infected with CNS when assistance during calving was needed. Udder edema prior to calving significantly decreased the odds of IMI with major pathogens. In treated heifers, no factors were detected that were associated with the likelihood of CNS IMI, whereas quarters from heifers were significantly more likely to be infected with major pathogens when they were housed in the calving pen >1 day, and when they had been in contact with the lactating cows prior to calving.

The risk factors for IMI that were identified in treated heifers were different than those in untreated heifers, independently from the pathogen group that was considered. It appears that prepartum treatment has not only changed the likelihood of infection but also the factors that are associated with infection. However, except for treated heifers with an IMI with major pathogens, only a small proportion of the variation could be explained in the final models. Therefore, other factors than those that were studied are explaining the likelihood of infection.

Introduction

A high proportion of dairy heifers freshen with IMI causing either clinical or subclinical mastitis (De Vliegher et al., 2012). A number of pathogens are involved, with CNS being the most prevalent in most studies (Fox, 2009). Intramammary infections caused by major pathogens in early lactating heifers are associated with elevated SCC in early lactation and result in milk production losses, udder health problems, and premature removal during the entire first lactation (De Vliegher et al., 2004, 2005a, 2005b; Piepers et al., 2009, 2010), stressing the need for effective control measures. On the contrary, CNS IMI in early lactating heifers have a less pronounced impact on the heifers' future performances, making the need for prevention of IMI with CNS at least in early lactating heifers not a priority, or even unwanted as heifers with CNS IMI at calving produce more and have a lower incidence of CM during their first lactation compared with non-infected heifers (Pearson et al., 2013; Piepers et al., 2010, 2013).

A number of factors increasing the odds of IMI in fresh dairy heifers have been identified (e.g. McDougall et al., 2009). A 10-point program specifically focusing on the prevention and control of heifer mastitis was proposed (De Vliegher et al., 2012), but did not discriminate between mastitis pathogen types. Still, studying pathogen group specific risk factors for IMI in early lactating heifers allows for the development of pathogen specific prevention and control programs.

Farmers are often more eager to treat animals, even when this includes off-label use of antimicrobials, than to improve their herd management (McDougall et al., 2009). However, in the light of prudent drug use, and even though prepartum treatment of heifers with antimicrobials is probably the easiest way to reduce the prevalence of IMI at calving on the short term (Nickerson, 2009), preventing herd health problems such as heifer mastitis via non-antibiotic strategies is preferable over adopting blanket treatment protocols to reduce the risk of antibiotic residues in foodstuff (e.g. milk) and the development of antimicrobial resistance in pathogens and commensals. This needs to be stressed since the long-term effects of prepartum antimicrobial treatment on farms suffering from heifer mastitis still remain uncertain (Borm et al., 2006; Sampimon et al., 2009; Passchyn et al., 2013). Logic suggests that temporary use of antimicrobials in control of a severe heifer mastitis problem can only be applied under strict conditions and when the etiology has been identified through culturing of milk samples, and should go along with the implementation of pathogen group specific preventive measures at the same time in order to further reduce the risk for new IMI (De Vliegher et al., 2012). Still, even when antimicrobial treatment is applied in the weeks before

calving, not all IMI will be cured nor prevented and it is currently not known which risk factors are associated with IMI in fresh heifers treated prior to calving with antimicrobials. We hypothesized that risk factors from treated heifers would be different from the ones in untreated heifers.

Materials and Methods

Herds and Animals

A clinical trial was conducted between September 2008 and June 2010 and included 229 heifers from 10 commercial, well-managed dairy herds, located in a radius of 20 km around the city of Torhout, province of West Flanders, Belgium. The trial was designed to assess both the short and long-term effects of a systemic prepartum therapy with penethamate hydriodide on udder health and milk production (Passchyn et al., 2013).

Information on herd size, BMSCC, heifer mastitis problems, and housing as well as the study design have been reported before (Passchyn et al, 2013). In short, before the actual trial was conducted, herds were first monitored by sampling the first eight heifers per herd that calved (80 heifers in total). After the 8th heifer had calved, monitoring of a herd ended and the actual clinical trial, comprising approximately an additional 16 heifers of which half were treated prior to calving and half served as untreated controls, started for this herd (Table 2). Heifers were alternately assigned by the first author based on their expected calving date; every other heifer that was expected to calve was treated with penethamate.

Table 2. Descriptive statistics of the number of herds, heifers and quarters included in the different analyses.

Dataset	Total number	Average[1]	Median	Range
Untreated heifers				
Herd	10	…	…	…
Heifer	73	7	8	6-8
Quarter	292	29	32	24-32
Treated heifers				
Herd	10	…	…	…
Heifer	76	8	8	6-8
Quarter	304	30	32	24-32

[1]Average number of heifers/quarters per herd

Composite milk samples were taken between 0 and 3 DIM for SCC measurement when no visual signs of CM were observed. Also, quarter milk samples were taken between 0 and 3 DIM for bacteriological culture both from quarters with and without signs of CM.

Sample Collection and Laboratory Analyses

Samples. All heifers were sampled by the first author once between 0 to 3 DIM (further referred to as early lactation) for bacteriological culture (5 mL; duplicate quarter milk samples), were checked for signs of CM, and sampled for determination of milk SCC if no signs were present (30 mL; samples of different quarters were combined into a composite sample using equal volumes). All milk samples were collected after disinfection of the teats and after the first streams of milk were discarded. Milk samples were immediately stored at 4°C and then transported under cooled conditions to the laboratory (Milk Control Centre Flanders, Lier, Belgium).

Bacteriological Culture. Bacteriological culture was done as previously described (Piepers et al., 2007). Briefly, 0.01mL of milk was plated on a blood-esculin agar (Oxoid, Erembodegem, Belgium; 1 plate per cow) and on MacConkey's agar (Oxoid; 1 plate per cow). All plates were incubated aerobically for 36 ± 12 h at 37 ± 1°C. A quarter was considered culture-positive when growth of ≥ 1 colony was detected. Samples yielding 3 or more different bacterial species were considered to be contaminated. Bacteria were identified by colony morphology and Gram-staining. For Gram-positive cocci, catalase tests were used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Colony morphology, hemolysis patterns, and DNase testing were used to distinguish *Staph. aureus* from CNS. Streptococci were subdivided into esculin-positive streptococci (*Streptococcus uberis*) and esculin-negative streptococci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*). Differentiation between *Streptococcus uberis* and other streptococci was done using bile aesculine agar and NaCl 6.5%. The Christie, Atkins, Munch-Petersen (CAMP) test was used to differentiate *Strep. agalactiae* from *Strep. dysgalactiae*. Coliforms including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. were differentiated from each other and from other Gram-negative bacteria based on the appearance on MacConkey's agar, KOH-testing, Triple Sugar Iron reactions, indol production and motility. *Staphylococcus aureus*, esculin-positive streptococci, *Strep. agalactiae*, *Strep. dysgalactiae* and coliforms were considered as major pathogens. Coagulase-negative staphylococci and *Corynebacterium bovis* were considered as minor pathogens.

Penicillin Resistance. All staphylococci were tested by the E-test method (AB BIODISK, Solna, Sweden), a stable-gradient agar diffusion technique that produces quantitative MIC results over a 15 $\log^2$ dilution range (Brown and Brown, 1991). Isolates were defined as "susceptible" to penicillin G when the MIC was ≤ 0.125 mg/L and as "resistant" to penicillin G when the MIC was > 0.125 mg/L, respectively (EUCAST, 2011).

Somatic Cell Count. Milk SCC was quantified by electronic counting using a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) at the Milk Control Centre Flanders (Lier, Belgium).

Data Collection

Herd-level Risk Factors. Binomial herd-level variables were constructed per herd using the data gathered from the monitoring heifers (n = 80) prior to the start of the actual clinical trial and were used as potential herd-level risk factors for IMI (Table 1).

Heifer-level Risk Factors. A number of potential heifer-level risk factors were recorded at the moment of sampling (0-3 DIM) (Table 1). Hygiene scores ranging from 1 (clean) to 5 (dirty) were assigned for four body areas: tail head, thigh, udder, and hind limbs (Hughes, 2001). As both udder hygiene score and leg hygiene scores are positively associated with IMI in lactating cows and as there exists a clear relationship between both hygiene scores (Schreiner and Ruegg, 2003), it was decided to combine them, rather than to evaluate them separately. Heifers with an average cleanliness score ≤ 2.0 (median value of all heifers) were considered as "clean" and heifers with a cleanliness score > 2.0 were considered as "unclean". Information on fly control on pasture and the deworming program for yearling heifers (yes/no) was obtained through a face-to-face interview. The applied fly control strategy was considered to be effective if heifers had been provided with two Flectron® ear tags (Zoetis, US) just before entering pasture or if during pasture season a heifer was treated with a pour-on solution registered for fly control in dairy cattle strictly in accordance with the manufacturer's directions. Body condition was scored approximately two wk before calving and at the moment of sampling (0-3 DIM) on a 5-point scale divided into quarter point increments (Edmondson et al., 1989). Ease of calving was assessed according to the following scale from 1 to 3: unassisted = heifer calved without any assistance; easy pull = one person without mechanical assistance; hard pull = ≥ 2 persons without mechanical assistance or 1 person with mechanical assistance. This variable was recoded for further analyses (0: no assistance/no difficulties; 1: assistance required). Udder edema around calving was scored between 0 and 3 DIM: 1 (no edema), 2 (slight edema), 3 (moderate edema), 4 (severe edema) or 5 (extremely severe edema) (Dentine and McDaniel, 1983). Recoding into a binary variable was applied for further analyses (0: no or slight edema; 1: moderate to extremely severe edema).

Table 1. Summary of all potential herd-, heifer- and quarter-level risk factors.

Independent variables	Recording method	Description/classification	Break down categories final models
Herd level[1]			
Average heifer milk SCC[2]	DHI[3]-records	arithmic average test-day SCC of the lactating heifers	> 150,000 versus ≤ 150,000 cells/mL
Average herd milk SCC[2]	DHI[3]-records	arithmic average test-day SCC of the lactating herd	> 200,000 versus ≤ 200,000 cells/mL
CNS monitoring heifers	Culture	percentage CNS infected quarters of monitoring heifers	≥ 18.5% versus < 18.5%[4]
CNS screening herd	Culture	percentage CNS infected quarters of lactating herd	≥ 7% versus < 7%[4]
Major monitoring heifers	Culture	presence of major pathogens in monitoring heifers	yes versus no
Major screening herd	Culture	presence of major pathogens in lactating herd	yes versus no
Presence of penicillin resistance	E-test	presence of resistance to penicillin in monitoring heifers	yes versus no
Hygiene herd	Visual	percentage of dirty monitoring heifers	≥ 37.5% versus < 37.5%[4]
Milk yield	DHI[3]-records	milk yield at first DHI-records of monitoring heifers	≥ 25.8 kg versus < 25.8 kg[4]
Heifer level			
Contact with lactating cows prior to calving	Interview	contact with lactating cows prior to calving	yes versus no
Supplementation with minerals/vitamins prior to calving	interview	whether or not the heifer was supplemented with minerals/vitamins prior to calving	yes versus no
Application fly control strategy	interview	effective fly control strategy[5]	yes versus no
Breed	DHI-records	2 different breeds	BW HF versus Red HF[6]
Calving pen time	interview	days in calving pen	≤ one day versus > one day[4]
Deworming program	interview	deworming program for yearling heifers	yes versus no
Difference in body condition score	visual and palpation	difference before versus after calving	< 0.5 versus ≥ 0.5 points
Ease of calving	visual and interview	assistance needed at calving	yes versus no
Fertilization method	interview	artificial insemination or bull	AI versus bull
Group prepartum	interview	animal group prior to calving	dry cows versus lactating cows versus end term heifers

Table 1. (continued).

Independent variables	Recording method	Description/classification	Break down categories final models
Heifer level			
Housing prepartum	interview	housing prior to calving	stable versus pasture
Hygiene score heifer	visual	thigh, udder and rear legs on 4-point scale	average hygiene score ≤ 2 versus > 2 points[5]
Milking prior to calving	interview	whether or not the heifer was milked prior to calving	yes versus no
Presence udder edema prior to calving	visual and palpation	udder edema prior to calving	yes versus no
Retentio secundinarum	interview and visual	retentio secundinarum	yes versus no
Season of calving	interview	month of calving	4 categories of 3 months each[7]
Supplementation with selenium	interview	whether or not the heifer was supplemented prior to calving	< 4mg/day versus ≥ 4mg/day[8]
Teat dipping prior to calving	interview	whether or not the teats were dipped prior to calving	yes versus no
Udder clipping prior to calving	interview	whether or not the udder was clipped prior to calving	yes versus no
Quarter level			
Quarter position	visual	position of the quarter	hind versus front quarter
Teat apex condition[9]	visual and palpation	no ring, smooth or slightly rough, rough, very rough	no ring versus smooth or slightly rough
Teat skin condition	visual	normal, dry, damaged or severly damaged	normal versus dry or damaged teat skin

[1]Binomial herd-level predictor variables constructed using data from monitoring heifers before the start of the actual clinical trial, [2]Somatic cell count, [3]Dairy Herd Improvement, [4]Threshold based on median value of study population, [5]Two Flectron® (Zoetis, US) ear tags just before having access to pasture or during pasture season treated strictly in accordance with the manufacturer's. prescriptions with a pour-on solution registered for fly control in dairy cattle, [6]Black and White Holstein Friesian and Red Holstein Friesian, [7]January – March, April – June, July – September, October – December, [8]Nutrient Requirements of Dairy Cattle, 2001, [9]The presence/absence of teat end lesions was recorded based on a visual scoring system (Neijenhuis et al., 2000), Additionally, the ring was classified as smooth (1) or rough (2). The scoring system was simplified as proposed by Mein et al. (2001).

Quarter-level Risk Factors. A number of potential quarter-level risk factors were recorded at the moment of sampling (0-3 DIM). The presence/absence of teat end lesions was recorded based on a visual scoring system (Neijenhuis et al., 2000) and expressed by the thickness of the callosity ring categorized into 5 classes: none (N), slight (A), moderate (B), thick (C), extreme (D). Additionally, the ring was classified as smooth (1) or rough (2).

The scoring system was simplified as proposed by Mein et al. (2001): no ring (N), smooth or slightly rough = a raised ring with no roughness or only mild roughness and no keratin fronds (1A, 1B, 1C or 2A), rough = a raised roughened ring with isolated fronds of old keratin extending 1-3 mm from the orifice (2B or 2C), very rough = a raised ring with rough fronds of old keratin extending > 4 mm from the orifice. The rim of the ring is rough and cracked giving the teat-end a “flowered” appearance (2D). None of the teats had a rough or very rough teat end. Scoring of teat skin condition was performed by means of palpation and visual inspection. Teat skin condition was classified as normal (i.e. smooth sheen, soft, healthy skin), dry (i.e. scaly, flaky or rough skin but without cracking), damaged (i.e. chapped with cracks) or severely damaged (i.e. deep chaps, scabs or open (ulcerative) lesions). The variable was recoded into a binary variable for further analyses (0: normal teat skin condition; 1: dry, damaged or severely damaged teat skin). None of the teats was severely damaged.

Definition of Intramammary Infection

A quarter was considered to have an IMI in early lactation (0 - 3 DIM) when the same mastitis pathogen was isolated from both duplicate milk samples (Borm et al., 2006). A quarter was considered as non-infected when no pathogens were isolated from both duplicate milk samples. When only in one sample from both duplicate milk samples a mastitis pathogen was isolated or when one or both milk samples were contaminated the data were considered missing.

Clinical mastitis was recorded by the first author and was defined as the presence of visual signs such as clots in the milk, with or without redness, swelling of the udder quarter, or systemic signs.

Statistical Analyses

Prior to statistical analysis, observations were explored and checked for unlikely values. No data were excluded for this reason. The dataset was divided in two subsets containing the data from the treated heifers and the data from the untreated control heifers, respectively. All analyses were performed on both subsets of data separately.

Logistic mixed regression models using 1st order marginal quasi-likelihood algorithms were fit in MLwiN 2.02.03 (Centre for Multilevel Modeling, Bristol, UK). Heifer and herd were included as random effects to correct for the clustering of quarters within heifer and heifers within herd. Statistical analyses were conducted with quarter as a unit of analysis.

Univariable associations between the two binary outcome variables (1) IMI with CNS versus non-infected at the quarter level (0= non-infected versus 1= infected with CNS), and (2) IMI with major pathogens versus non-infected at the quarter level (0= non-infected versus 1= infected with a major pathogen), respectively, and all independent variables at the herd, heifer and quarter level were tested. Statistical significance in this step was assessed at $P < 0.20$. Secondly, Pearson and Spearman correlation coefficients were calculated among the significant independent variables to check for multicollinearity. If two independent variables had a correlation coefficient ≥ 0.6, only the one with the highest statistical significance was selected for further analysis. None of the variables had to be omitted because of this reason. In the third step, multivariable models were fit with statistical significance in this step set at $P < 0.05$. A variable was considered to act as a confounder if its removal made the regression coefficients of the remaining variables undergo a relative change > 25% or in case the regression coefficient ranged between -0.4 and 0.4, if an absolute change > 0.1 was observed (Noordhuizen et al., 2001). Finally, all first-order interactions among the remaining independent variable in the multivariable models were tested and removed when non-significant (Wald's tests $P > 0.05$). The fit of the final models was evaluated by the inspection of the quarter level standardized residuals plotted against the normal scores and against the quarter level predicted values, and by calculation of the deviance chi-square statistic to the remaining degrees of freedom (Dohoo et al., 2009). The Hosmer Lemeshow goodness-of-fit measure and the sensitivity and specificity of the model were assessed on the fixed effect models only (Dohoo et al., 2009) using SAS 9.3 (PROC LOGISTIC, SAS Institute Inc., NC, USA).

The proportion of variation for IMI with CNS and IMI with major pathogens present at the herd, heifer and quarter level for both the null and the final models was estimated by assuming that the total variance at the quarter level on the logit scale was $\pi^{2/3}$ with $\pi = 3.1416$ (Dohoo et al., 2001). Using this approach, the total variance was estimated as followed:

$$\text{Var}(Z_{ijk}) = \text{var}(\mu_{Herd(j)}) + \text{var}(\mu_{Heifer(i)}) + \text{var}(\varepsilon_{ijk}) = \sigma^2_{Herd} + \sigma^2_{Heifer} + \pi^2/3$$

where $\pi^2/3$ = variance occurring at the quarter level,
σ^2_{Heifer} = variance occurring at the heifer level,
and σ^2_{Herd} = variance occurring at the herd level.

Table 3a. Significant ($P \leq 0.20$) unconditional associations at the herd, heifer and quarter level for IMI with coagulase-negative staphylococci (CNS) in untreated and treated heifers based on logistic mixed regression models (See Table 1 for details on the different independent variables)**.**

	IMI with CNS					
	Untreated heifers			Treated heifers		
Independent variables	N[1]	%[2]	*P*-value	N	%[1]	*P*-value
Herd level						
Average heifer milk SCC			0.200			NS
>150,000 cells/mL	84	19.0		…	…	
≤150,000 cells/mL	130	29.2		…	…	
CNS screening herd			NS			0.146
≥18.5%	…	…		94	23.4	
<18.5%	…	…		133	13.5	
Heifer level						
Hygiene score of the heifer			NS			0.080
clean	…	…		182	20.3	
unclean	…	…		45	6.7	
Deworming program			NS			0.093
yes	…	…		83	25.3	
no	…	…		144	13.2	
Ease of calving			0.005			NS
assistance	41	48.8		…	…	
no assistance	173	19.6		…	…	
Group prepartum			NS			0.085
dry cows	…	…		129	23.2	
lactating cows	…	…		30	3.3	
end term heifers				68	13.2	
Housing prepartum			0.049			NS
stable	163	20.9		…	…	
pasture	51	39.2		…	…	
Season of calving			NS			0.064
January – March	…	…		71	14.1	
April – June	…	…		25	28.0	
July – September	…	…		61	24.9	
October - December	…	…		70	11.4	
Supplementation with selenium			0.028			NS
≥ 4mg/day	67	13.4		…	…	
< 4mg/day	147	30.6		…	…	
Quarter level						
	…	…	…	…	…	…

[1]Total number of quarters, [2]Percentage of quarters infected with CNS.

Results

Descriptive Results

In total, 149 heifers (596 quarters), of which 76 treated heifers (304 quarters) and 73 untreated heifers (292 quarters), were included in the trial (Passchyn et al., 2013).

Overall, in early lactation 347 quarters were non-infected, 34 quarters (5.7%) were infected with a major pathogen [14 (2.3%) in treated heifers and 20 (3.4%) in untreated heifers, respectively] and 94 quarters (15.8%) with CNS [40 (6.7%) in treated heifers and 54 (9.1%) in untreated heifers, respectively]. Within the major pathogens, *Staphylococcus aureus* (n = 6), *Streptococcus uberis* (n = 10), *Streptococcus dysgalactiae* (n = 3), other *Streptococci* (n = 11) and *Escherichia coli* (n = 4) were isolated. Milk samples from 37 quarters were contaminated and for 84 quarters assignment of an IMI status was not possible, because in only one sample from both duplicate samples a mastitis pathogen could be isolated (data encoded as missing values). In addition, six quarters from six heifers in the treatment group showed signs of CM in early lactation (4%). *Streptococcus uberis* (n = 1), *Corynebacterium bovis* (n = 1), *Staphylococcus aureus* (n = 1) were isolated; one sample was contaminated and two samples remained culture negative.

Risk Factors for IMI in Untreated Heifers

Four and two risk factors, respectively, were unconditionally associated ($P \leq 0.20$) with IMI with CNS and IMI with major pathogens in untreated heifers (Table 3a and 3b).

The final models revealed that quarters from untreated heifers that were supplemented with selenium prepartum (≥ 4 mg/day) were significantly less likely to be infected with CNS whereas when assistance during calving was needed, quarters were significantly more likely to be infected with CNS (Table 4). Udder edema prior to calving significantly decreased the odds of IMI with major pathogens (Table 4).

Model sensitivity (ability to predict occurrence of IMI) was 49.2% for the model with IMI with CNS and 43.5% for the model with IMI with major pathogens. Model specificity (ability to predict non-occurrence of IMI) was 84.1% and 90.9%, respectively. The Hosmer-Lemeshow test for the final model with IMI with CNS was not statistically significant ($P = 0.25$). The deviance chi-square statistic per degree of freedom was 1.05 for the model based on IMI with CNS and 0.95 for the model based on IMI with major pathogens, indicating a good model fit.

Risk Factors for IMI in Treated Heifers

Five and four risk factors, respectively, were unconditionally associated (P ≤ 0.20) with IMI with CNS and IMI with major pathogens in treated heifers (Table 3a and 3b).

For IMI with CNS, no risk factors were detected (Table 4). In the final model for IMI with major pathogens, two risk factors were significant: quarters were significantly more likely to be infected with major pathogens if a heifer was housed in the calving pen for more than one day, and if a heifer had been in contact with the lactating cows prior to calving (Table 4).

Table 3b. Significant (*P* ≤ 0.20) unconditional associations at the herd, heifer and quarter level for IMI with major pathogens in untreated and treated heifers based on logistic mixed regression models (See Table 1 for details on the different independent variables)**.**

	IMI with major pathogens					
	Untreated heifers			Treated heifers		
Independent variables	N[1]	%[2]	*P*-value	N	%[1]	*P*-value
Herd level						
Average herd milk SCC			NS			0.083
<200,000 cells/mL	…	…		133	9.8	
≥200,000 cells/mL	…	…		68	1.5	
Heifer level						
Calving pen time			NS			0.006
≤ one day	…	…		120	3.3	
> one day	…	…		81	12.3	
Contact with lactating cows prior to calving			NS			0.105
yes	…	…		44	16.0	
no	…	…		155	4.5	
Udder edema prior to calving			0.019			NS
yes	91	4.4		…	…	
no	89	18.0		…	…	
Fertilization method			0.029			NS
artificial insemination	154	7.8		…	…	
bull	26	30.1		…	…	
Group prepartum			NS			0.116
dry cows	…	…		102	2.9	
lactating cows	…	…		36	19.4	
end term heifers	…	…		63	6.3	
Quarter level						
	…	…	…	…	…	…

[1]Total number of quarters, [2]Percentage of quarters infected with major pathogens.

Table 4. Final multivariable, logistic mixed regression models for IMI with coagulase-negative staphylococci (CNS) and major pathogens, respectively, in treated and untreated heifers.

Outcome variable	Independent variable	Untreated heifers				Treated heifers			
		β[1]	SE[2]	OR[3]	95% CI[4]	β	SE	OR	95% CI
IMI with CNS									
	Supplementation of selenium[5] (≥4mg/day versus <4mg/day)[5]	-1.047	0.475	0.35	0.14-0.89	…	…	…	…
	Assistance at calving[5] (yes versus no)[5]	1.379	0.469	3.97	1.58-9.96	…	…	…	…
IMI with major pathogens									
	Calving pen (>1 day versus ≤1 day)[5]	…	…	…	…	2.529	0.793	12.54	2.65-59.34
	Contact with lactating cows prior to calving (yes versus no)[5]	…	…	…	…	1.806	0.893	6.09	1.06-35.03
	Udder edema prior to calving[5] (yes versus no)	-1.594	0.679	0.20	0.05-0.77	…	…	…	…

[1]Regression coefficient, [2]Standard error of the mean, [3]Odds Ratio, [4]95% Confidence Interval, [5]Heifer-level risk factor.

For the final model with IMI with major pathogens, model sensitivity (ability to predict occurrence of IMI) and specificity (ability to predict non-occurrence of IMI) were 64.5 and 86.8%, respectively. The Hosmer-Lemeshow test was not statistically significant (P = 0.93). The deviance chi-square statistic per degree of freedom was 0.99, indicating a good model fit.

Table 5a. Variance components at the herd, heifer and quarter level of the null models and the final multivariable models for IMI with coagulase-negative staphylococci (CNS) in untreated heifers and treated heifers.

	Data hierarchy	**IMI with CNS**			
		Untreated heifers		Treated heifers	
		Var.Est.[1] ± SE[2]	%	Var.Est.[1] ± SE[2]	%
Null models					
	Herd	0	0	0	0
	Heifer	1.036 ± 0.467	23.9	1.486 ± 0.614	31.1
	Quarter	3.289[3]	76.1	3.289[3]	68.9
	Total Variance	4.387	100.0	4.776	100.0
Final multivariable models					
	Herd	0	0	0	0
	Heifer	0.722 ± 0.437	18.0	1.486 ± 0.614	31.1
	Quarter	3.289[3]	82.0	3.289[3]	68.9
	Total Variance	4.308	100.0	4.776	100.0

[1]Variance estimate, [2]Standard error, [3]$\pi^2/3$ = variance occurring at the quarter level (Dohoo et al., 2001).

Variance Components

The variance components for the different models are presented in Table 5a and 5b. For the null model of CNS IMI in treated and untreated heifers, 31.1% and 23.9% of the variation resided at the heifer level, respectively. For IMI with major pathogens in treated and untreated heifers this was 54.3% and 41.9%. No variation resided at the herd level in the models with CNS IMI as outcome, whereas some variation resided at this level in the models with IMI with major pathogens as outcome variable.

Table 5b. Variance components at the herd, heifer and quarter level of the null models and the final multivariable models for IMI with major pathogens in untreated heifers and treated heifers.

	Data hierarchy	IMI with major pathogens			
		Untreated heifers		Treated heifers	
		Var.Est.1 ± SE²	%	Var.Est.1 ± SE²	%
Null models					
	Herd	0	0	0.245 ± 0.776	3.2
	Heifer	2.369 ± 1.049	41.9	4.201 ± 1.762	54.3
	Quarter	3.289[3]	58.1	3.289[3]	42.5
	Total Variance	5.761	100.0	7.736	100.0
Final multivariable models					
	Herd	0.211 ± 0.545	4.0	1.671 ± 1.216	30.4
	Heifer	1.719 ± 1.050	33.0	0.537 ± 0.914	9.8
	Quarter	3.289[3]	63.0	3.289[3]	59.8
	Total Variance	5.216	100.0	5.498	100.0

[1]Variance estimate, [2]Standard error, [3]$\pi^2/3$ = variance occurring at the quarter level (Dohoo et al., 2001).

Discussion

To our knowledge, this is the first study investigating pathogen group specific risk factors for IMI in both treated and untreated heifers participating in a prepartum antimicrobial treatment trial. Treated and untreated heifers were part of the same management when housed at the same farm, allowing us to compare risk factors in play besides determining the variance components.

When looking at the unconditional associations, as well as at the final models, the risk factors for IMI that were identified in treated heifers were different than those in untreated heifers, independently from the pathogen group that was considered. It looks as if prepartum treatment has not only changed the likelihood of infection (Passchyn et al., 2013) but also the factors that are associated with infection. This suggests that in herds where prepartum treatment is implemented, prevention should focus on other aspects than in herds where no such programs are in place, although this needs to be substantiated in future research.

As no other studies have reported on risk factors for IMI in heifers that were treated with antimicrobials before calving, we limit ourselves to comparing the factors associated with the IMI likelihood found in the untreated control heifers with those reported in the scientific literature. The fact that moderate to excessive edema prior to calving was negatively associated with the likelihood of IMI with major pathogens in untreated control heifers was unexpected and was in contrast with previous findings (Slettbakk et al., 1995; Waage et al., 2001; Compton et al., 2007). Actually, we have also reported before that udder edema prior to calving was positively associated with the likelihood of IMI in heifers, with contagious major pathogens to be more specific but not with the likelihood of IMI with environmental major pathogens (Piepers et al., 2011). Because of the limited number of IMI with major pathogens (n = 20) in the current study, stratification into environmental and contagious major pathogens was not possible.

Selenium supplementation (≥ 4mg/day) was negatively associated with the likelihood of CNS IMI in control heifers, somewhat contrasting with the findings of a previous study (Piepers et al., 2011) in which no association could be found between Se supplementation before calving and the likelihood of CNS IMI. Since CNS is a large group consisting of different species together with the fact that species seem to behave differently (Supré et al., 2011; Piessens et al., 2012) and since the CNS isolates from the current study were not identified to the species level, we could not further elaborate this finding. Still, it can be anticipated that different species were involved explaining the contradictions between studies. Anyway, it underlines the need to study risk factors for CNS species-specific IMI but this needs large studies and datasets (e.g. Reyher et al., 2011), also larger than the one that is presented in this article.

Observational studies have suggested that dystocia is associated with a higher risk of CM in heifers within a month of calving compared with eutocia (Oltenacu and Ekesbo, 1994; Barnouin and Chassagne, 2001; Svensson et al., 2006). Our study confirms these findings as quarters belonging to heifers undergoing dystocia were more likely to have CNS IMI than quarters from heifers where no assistance was required during calving. Still, the majority of cases of IMI in our study were not associated with clinical signs, as expected because CNS are not likely to cause CM (Supré et al., 2011; Hertl et al., 2014), which is a substantial difference. Svensson et al. (2006) hypothesized that the association between reproductive disorders and CM could be due to a common factor affecting both disease complexes. Based on the conclusions of Proudfoot et al. (2009) showing that cows with dystocia had reduced DMI and water intake 24h before calving, a more pronounced negative energy balance in heifers that needed assistance followed by decreased weakened immunity, could possibly

explain this finding. Still, it does not explain why this factor was not associated with IMI with major pathogens.

Quarters from treated heifers staying in the calving pen for more than one day were more likely to be infected with major pathogens. As almost 50% of the farms in Flanders use their calving pen for both sick and periparturient cows (De Vliegher et al., 2004), we hypothesize a prolonged stay in a more infectious environment explains this finding. In our study as well, 8 out of 10 farmers used their calving pen for both sick and periparturient cows.

Except in the model for IMI with major pathogens in treated heifers, all variation in the likelihood of IMI with both CNS and major pathogens occurred at the heifer and quarter level and none at the herd level. This coincides with our previous findings studying early lactation SCC in heifers (De Vliegher et al., 2004). Approximately 97% of the variance in that outcome variable occurred at the heifer level, with very small variance estimates at the herd level. At that time we suggested that focusing on heifers rather than on the herds seems to be necessary when dealing with heifer mastitis on the short term, and this seems to be the case also when studying IMI. However, as the variation residing at the herd level for IMI with major pathogens was higher in the treated heifers compared to the untreated, we speculate that some herds benefit more from treatment than others. It is indeed well established that treatment of mastitis is more effective in well-managed than in poorly-managed herds where cured cows or heifers are more likely to become re-infected (Barlow et al., 2009).

The heifer-level variation for both IMI with CNS and IMI with major pathogens was more pronounced in treated heifers than in untreated heifers. Although we are considering two different datasets making comparison difficult or even unwanted, we hypothesize that some heifers benefit more from treatment than others, especially heifers with an IMI with major pathogens. This hypothesis is supported by two other studies in which both the average cure rate (Borm et al., 2006) and the number of infected quarters (Sampimon et al., 2009 and Passchyn et al., 2013) of prepartum treated heifers differed between both herds and heifers within herds. Still, the lack of data on cure rates in our study makes it difficult to further substantiate this hypothesis.

The fact that most of the variation in IMI with CNS resided at the quarter level and in IMI with major pathogens at the heifer level, can probably be explained by the fact that quarters are more likely to be infected with CNS than with a major pathogen. In this study 15.8 % of the quarters were infected with CNS and 5.7% with a major pathogen, prevalences that are in line with those from literature (Fox, 2009; De Vliegher et al., 2012). The fact that when cows

have an IMI with CNS, often more than one quarter per cow is infected with CNS has been described before (Piepers et al., 2007).

Except for treated heifers with an IMI with major pathogens, only a small proportion of the variation in the outcome variables was explained in the final multivariable models. This indicates that different factors than those we have studied are explaining the likelihood of infection (e.g. anatomy of the teat, genetic background of heifer, young stock management). The same finding (De Vliegher et al., 2004) actually urged us to study the genetic background of heifer mastitis and by focusing on the CXCR1 gene, we recently described a susceptibility in heifers that was different for CNS compared with major pathogens (Verbeke et al., 2012). In treated heifers, avoiding contact with lactating cows prior to calving and keeping the fresh heifers in the calving pen only for a short period of time (< 1 day) seems to be important measures to reduce the (re)infection risk. In general and at the same time, additional unstudied risk factors on the herd level must be further explored (e.g. barn design, feeding, etc.).

Conclusions

Quarters from untreated heifers supplemented with ≥ 4 mg selenium/day prepartum were significantly less likely to be infected with CNS whereas quarters were more likely to be infected with CNS when assistance during calving was needed. Udder edema prior to calving was negatively associated with the likelihood of IMI with major pathogens. In treated heifers no factors were detected that were associated with the likelihood of CNS IMI, whereas quarters from heifers were significantly more likely to be infected with major pathogens when it was housed in the calving pen >1 day, and when it had been in contact with the lactating cows prior to calving. Strikingly, all identified risk factors for IMI in treated heifers, also in the univariable analyses, were different than those in untreated heifers, indicating that prepartum systemic antimicrobial treatment not only change the likelihood of infection but also the factors that are associated with infection. Factors other than those we have studied are explaining the likelihood of infection as only a small proportion of the variation was explained in the final models, except for the one describing the likelihood of major pathogen IMI in treated heifers. More and different risk factors at the quarter (e.g. anatomy of the teat), heifer (e.g. genetics, immunity), and at the herd level (e.g. barn design, nutrition) should be included in future research.

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References

Barlow, J. W., L. J. White, R. N. Zadoks, and Y. H. Schukken. 2009. A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds. Prev. Vet. Med. 90:31-42.

Barnouin, J., and M. Chassagne. 2001. Predictive variables for the occurrence of early clinical mastitis in primiparous Holstein cows under field conditions in France. Can. Vet. J. 42:47-53.

Borm, A. A., L. K. Fox, K. E. Leslie, J. S. Hogan, S. M. Andrew, S. P. Oliver, Y. H. Schukken, D. D. Hancock, C. T. Gaskins, W. E. Owens, and C. Norman. 2006. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. J. Dairy Sci. 89:2090-2098.

Brown D. F. J., and L. Brown. 1991. Evaluation of the Etest: a novel method of quantifying antimicrobial activity. J. Antimicrob. Chemother. 27:185–190.

Compton, C. W. R., C. Heuer, K. Parker, and S. McDougall. 2007. Epidemiology of mastitis in pasture-grazed peripartum dairy heifers and its effects on productivity. J. Dairy Sci. 90:4157-4170.

Dentine, M. R., and B. T. McDaniel. 1983. Variation in edema scores from herd-year, age, calving month and sire. J. Dairy Sci. 66:2391-2399.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Impact in dairy heifers of early lactation somatic cell count on somatic cell counts during the first lactation. J. Dairy Sci. 87:3672-3682.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2005a. Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. J. Dairy Sci. 88:938-947.

De Vliegher, S., H. W. Barkema, G. Opsomer, A. de Kruif, and L. Duchateau. 2005b. Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. J. Dairy Sci. 88:569-576.

De Vliegher, S., L. K. Fox, S. Piepers, S. McDougall, and H. W. Barkema. 2012. Invited review: mastitis in dairy heifers. J. Dairy Sci.. 95:1025-1040.

Dohoo, I. R., E. Tillard, H. Stryhn, and B. Faye. 2001. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. Prev. Vet. Med. 50:127-144.

Dohoo, I. R., Martin, S. W., and H. Stryhn. 2009. Mixed models for discrete data. Pages 584-585 in Veterinary Epidemiologic Research. Vol. 2. I. Dohoo, W. Martin and H. Stryhn, ed. AVC Inc., Charlottetown, Canada.

Edmondson, A. J., I. L. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 94:250-261.

EUCAST. 2011. Clinical Breakpoint Table. http://www.eucast.org/antimicrobial_susceptibility_testing/previous_versions_of_tables/. Accessed June 1, 2012.

Fox, L. K. 2009. Prevalence, incidence and risk factors of heifer mastitis. Vet. Microbiol. 134:82-88.

Hertl, J. A., Y. H. Schukken, F. L. Welcome, L. W. Tauer, and Y. T. Gröhn. 2014. Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. J. Dairy Sci. 97:1-16.

Hughes, J. 2001. A system for assessing cow cleanliness. In Pract. 23:517-524.

Mein, G. A., F. Neijenhuis, W. F. Morgan, D. J. Reinemann, J. E. Hillerton, J. R. Baines, I. Ohnstad, I., M. D. Rasmussen, L. Timms, L., J. S. Britt, R. Farnsworth, N. B. Cook, and T. Hemling. 2001. Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors. Pages 347-351 in Proc. 2nd Int. Symp. on Mastitis and Milk Quality, Vancouver, Canada.

McDougall, S., K. I. Parker, C. Heuer, and C. W. R. Compton. 2009. A review of prevention and control of heifer mastitis via non-antibiotic strategies. Vet. Microbiol. 134:177-185.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. d. National Research Council, Washington, DC.: National Academy Press.

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

Noordhuizen, J. P. T. M., K. Frankena, M. V. Thrusfield, and E. A. M. Graat. 2001. Application of quantitative methods in veterinary epidemiology. Wageningen Press, Wageningen, The Netherlands.

Oltenacu, P. A., and I. Ekesbo. 1994. Epidemiological study of clinical mastitis in dairy cattle. Vet. Res. 25:208-212.

Passchyn, P., S. Piepers, and S. De Vliegher. 2013. Systemic prepartum treatment of end-term dairy heifers with penethamate hydriodide: effect on udder health, milk yield and culling until 120 days in milk. J. Dairy Sci. 96:6324-6335.

Pearson, L. J., J. H. Williamson, S. A. Turner, S. J. Lacy-Hulbert, and J. E. Hillerton. 2013. Peripartum infection with Streptococcus uberis but not coagulase-negative staphylococci reduced milk production in primiparous cows. J. Dairy Sci. 96:158-164.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H. W. Barkema, and S. De Vliegher. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res. 74:478-483.

Piepers, S., S. De Vliegher, A. de Kruif, G. Opsomer, and H. W. Barkema. 2009. Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. Vet. Microbiol. 134:113-120.

Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than non-infected heifers. J. Dairy Sci. 93:2014-2024.

Piepers, S., K. Peeters, G. Opsomer, H. W. Barkema, K. Frankena, S. De Vliegher. 2011. Pathogen group specific risk factors at herd, heifer and quarter levels for intramammary infections in early lactating dairy heifers. Prev. Vet. Med. 99:91-101.

Piepers, S., Y. H. Schukken, P. Passchyn, S. De Vliegher. 2013. The impact of intramammary infection with coagulase-negative staphylococci in early lactating heifers on milk yield throughout first lactation revisited. J. Dairy Sci. 96:5095-5105.

Piessens, V., S. De Vliegher, B. Verbist, G. Braem, A. Van Nuffel, L. De Vuyst, and E. Van Coillie. 2012. Intra-species diversity and epidemiology varies among coagulase-negative Staphylococcus species causing bovine intramammary infections. Vet. Microbiol. 155(1):62-71.

Proudfoot, K. L., J. M. Huzzey, and M. A. G. von Keyserlingk. 2009. The effect of dystocia on the dry matter intake and behavior of Holstein cows. J. Dairy Sci. 92:4937-4944.

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.

Sampimon, O. C., S. De Vliegher, H. W. Barkema, J. Sol, and T. J. G. M. Lam. 2009. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. J. Dairy Sci. 92:4395-4403.

Sampimon, O. C., H. W. Barkema, I. M. G. A. Berends, J. Sol, and T. J. G. M. Lam. 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134:37-44.

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

Slettbakk, T., A. Jorstad, T. B. Farver, and J. C. Holmes. 1995. Impact of milking characteristics and morphology of udder and teats on clinical mastitis in first-lactation and 2nd – lactation Norwegian cattle. Prev. Vet. Med. 24:235-244.

Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De Vliegher. 2011. Some coagulase-negative Staphylococcus species affect udder health more than others. J. Dairy Sci. 94:2329-2340.

Svensson, C., A.-K. Nyman, K. Persson-Waller, and U. Emanuelson. 2006. Effects of housing, management, and health of dairy heifers on first-lactation udder health in southwest Sweden. J. Dairy Sci. 89:1990-1999.

Verbeke, J., S. Piepers, L. Peelman,M. Van Poucke, and S. De Vliegher. 2012. Pathogen-group specific association between CXCR1 polymorphisms and subclinical mastitis in dairy heifers. J. Dairy Res. 79:341-351.

Waage, S., S. A. Odegaard, A. Lund, S. Brattgjerd, and T. Rothe. 2001. Case control study for risk factors for clinical mastitis in postpartum dairy heifers. J. Dairy Sci. 84:392-399.

Chapter 6

General Discussion

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Introduction

The general aim of this thesis was to study (new) challenges related to udder health and milk quality (e.g. prudent use of antimicrobials, milk quality and safety, animal welfare, emerging pathogens, volatile milk prices, pressure to grow) that dairy farmers and their veterinarians are currently facing or will be facing in the (near) future. We believe the results from this thesis indeed give better insights in which bottlenecks need to be first unblocked and might, in turn, offer opportunities for both the farmer and his veterinarian by implementing fixes for them through veterinary herd health management (VHHM) programs (Figure 5) . We came to a number of conclusions of which the most important are:

- Only one-third of the Flemish dairy herds were actually able to combine a low BMSCC with a low estimated incidence of clinical mastitis (EICM) in 2009; part of the variation in the occurence of CM and in the BMSCC was explained by differences in (udder health) management practices (*Chapter 3.1*).
- *Mycoplasma bovis* is also threatening udder health on Flemish dairy farms as the herd-level prevalence of *Mycoplasma bovis* in bulk milk was estimated to be at least 1,5% (*Chapter 3.2*); this is worrying, especially so because an increasing herd size is a determining factor.
- The variation in BC and CC in unpasteurized bulk milk in Flemish dairy herds is related to different factors than the ones related to milking, herd health and dry cow management (*Chapter 4.1*).
- Only 52.9% of the dairy herds achieved their MQP in all 12 months in 2009; the main reason for not achieving the MQP was an elevated CC (*Chapter 4.2*).
- Systemic use of penethamate hydriodide in heifers prior to calving results in levels of penicillin G in mammary tissue and secretion substantially higher than the MIC_{90} of pathogens associated with heifer mastitis (*Chapter 5.1*).
- When looking at short- and long-term effects, herds with a low prevalence of heifer mastitis are not likely to benefit from prepartum systemic antimicrobial treatment of end-term heifers (*Chapter 5.2*).
- Finally, pathogen group-specific risk factors for intramammary infection in heifers participating in a prepartum antimicrobial treatment trial were different between treated heifers and untreated control heifers (*Chapter 5.3*), suggesting that prepartum treatment not only changes the likelihood of infection, but also the factors that are associated with infection.

First, some insights are discussed on how udder health and milk quality on the Flemish dairy herds can be further improved in order to better respond to the public concerns and the volatile and often low milk prices and which bottlenecks need to be first unblocked in order to do so. Second, the profitability of a better udder health and milk quality on an average dairy farm will be simulated. Next, some aspects on the emergence of antimicrobial resistance and the possible indirect effects of antimicrobial treatment are discussed. To end this thesis, some challenges and opportunities for the herd health advisor are pinpointed.

Udder Health

Situation in Flanders

Despite the fact that much research and effort has been dedicated to mastitis prevention and control, it remains a persistent problem and the most expensive disease of dairy cows (Schepers and Dijkhuizen, 1991), also in Flanders, Belgium. Since the disease has two very distinctive presentations (subclinical and clinical) that are both of importance, we opted to combine them in our definition of herds having a "good udder health" (in *Chapter 3.1*). Actually, a weak correlation was found between EICM and BMSCC, stressing the importance of combining both parameters to precisely picture, monitor and evaluate udder health on dairy farms: herds with a low BMSCC might well be suffering from CM and vice versa. Surprisingly, only one-third of the herds combined a low BMSCC (< 250,000 cells/mL) with a low EICM (< 3%). The thresholds we used to define the different strata were yet not at all that strict. Lowering the threshold to 200,000 cells/mL for BMSCC and < 2% for EICM which are arguably better thresholds from an economical point of view, would have resulted in only 8.3% of the herds in our study achieving the status of having a good udder health. These findings are worrying and indicate that there is still a lot of room for improvement and lots of opportunities for both producers and their advisors.

The first standard mastitis control program comprised 5 points as mentioned before: appropriate treatment of CM, culling of chronically infected cows, postmilking teat disinfection, correct maintenance and use of the milking equipment, and application of blanket dry cow therapy (Neave et al., 1969). Surprisingly, we had to conclude that these and other well-known and effective measures are not always implemented on Flemish dairy farms. For example, still 9% of the herds is not applying postmilking teat disinfection, and 55% of the herds is not keeping their cows standing after milking in headlocks, although possible. On 55% of the herds the calving pen was also used as a sick pen and 27% of the herds is not providing dry cow minerals to their dry cows. One of the most striking findings was that 90% of the herds was not

replacing the teat liners on time. These findings probably explain why only a minority of Flemish dairy herds has a good udder health. Herd health advisors need to be aware of the fact that technical knowledge alone is not enough to be successful. To be succesful, knowledge should be used and thus should the farmer be motivated to comply with some basic udder health concepts. Dutch research has clearly demonstrated that veterinarians are perceived by farmers as an important and highly respected information source with regard to udder health, but that they often lack the skills to sufficiently motivate the farmer to take action (Jansen et al., 2008; Lam et al., 2011). An upgrade of the veterinarian's communication and motivation skills could thus further strengthen their position as herd health consultants, and boost the transfer of (their) knowledge to the field.

We acknowledge our work was not designed to accurately determine the actual incidence rate of clinical mastitis (IRCM). Data were collected through an online questionare (with a response rate of 24%) which is not the best manner to get information on disease occurrence. Still, only 33% of the responding farmers indicated they kept records of all cases of CM. This is minimal and disappointing. Also, we realise BMSCC is only a proxy of the herd-level prevalence of cows with subclinical mastitis as only those cows of which the milk is delivered contribute to the BMSCC (Lievaart et al., 2007). The average EICM was 3.9% per month or 46.8% EICM per year, which is considered to be very high. The latter information triggered our research group to estimate the IRCM more precisely in a follow-up study specifically designed to do so (Verbeke et al., 2014). Eventually an IRCM of 26% was reported which is lower than the numbers included in this thesis (46.8%). Nevertheless, based on the results of Verbeke et al. (2014), still 78,000 first cases of CM per year occur which is immense if you consider the associated economic losses, the impact on animal welfare and the related use of antimicrobials with the latter two being growing public concerns. Using a calculated cost of CM of € 210 per case (Huijps et al., 2008), CM alone results in an overall cost for the Flemish dairy industry of more than € 16,380,000 per year.

In the study of Verbeke et al. (2014), farms were randomly selected and 79 % of the targeted producers agreed to participate and record all CM cases, but nothing was mentioned on whether farmers already had good record keeping in place before the trial started. Interestingly, Peeler et al. (2000) reported that farmers that kept records of CM reported a significantly higher level of mastitis compared to those not keeping records which is in contrast with the findings of our study and the one of Verbeke et al. (2014). In the latter study, a financial incentive of €3 was paid per collected sample of CM. Since a CM case has a calculated cost of €210 (Huijps et al., 2008) and €3 is not a lot of money, this incentive did probably not help to collect and record every single case, most probably still resulting in an underestimation of the true IRCM. Also, farmers were contacted every 2 months by phone, the herd veterinarian was actively involved and 2 visits were done by the first author during the trial. These contacts

might have biased the results, because extra attention was paid to udder health on those farms, making it more likely that farmers improved their (udder health) management during the study resulting in a lower incidence of CM. According to Barkema et al. (1999) and based on personal experiences, farmers with good udder health management have better record-keeping systems and pay more attention to individual cows. Since in our study a majority of farmers did not keep good records of CM, we probably selected for ”underperforming managers”, expressed by a higher EICM or we can say that only a minority of farmers in Flanders is keeping good records of CM. This is a bit striking as the larger herd size of the participating farmers in our questionnaire compared with the average Flemish herd size and the need to have internet access, made us believe we selected more progressive farmers. Altogether, it is likely that the true IRCM in Flanders is somewhere in-between the values obtained by Verbeke et al. (2014) and our results. Also, in both studies no difference was made between a first case of mastitis and a recurrent case. This difference would however be very helpful in interpreting the CM data and clarifying the etiology of infection. Cows with histories of previous cases of CM are less likely to respond to therapy (Pinzon-Sanchez et al., 2010). Before initiating mastitis treatments, producers should consider this effect and enroll a culling strategy on their farm for those cows suffering from recurrent cases.

Record-keeping of clinical and subclinical mastitis

A first bottleneck that needs to be unblocked is the substandard record keeping of clinical and subclinical mastitis in Flanders (Figure 5). Based on the results obtained via our questionnaire and personal experiences, it is clear that only a minority of the farmers is recording CM cases on their farm. This is quite surprising since mastitis is known to be the most costly disease and record keeping of antimicrobial treatments is mandatory by the Belgian Dairy Quality Assurance Program (IKM). Also, on 33% of the farms, treatment decisions were ‘sometimes to never’ based on bacteriological cultures. In more than 40% of the Flemish dairy herds, culturing of CM cases is actually never performed. As a consequence, only 40% of the farms is evaluating the CM treatments “often”. In times that prudent use of antimicrobial drugs has become a priority, this finding is astonishing. Milk recording data (i.e. test-day milk production and composite milk SCC) is collected at an interval of 4 to 6 weeks in approximately 40% of the Flemish herds (personal communication, Benny Declerck, CRV). Detection of cows with sublinical mastitis is, as a consequence, complicated in the majority of the herds in Flanders in which BMSCC has to be used as a proxy for the prevalence of cows with subclinical mastitis. We should also be aware of the fact that non-saleable milk (e.g. milk from treated cows, milk from cows with a high cell count) remains an unknown component in that respect (Lievaart et al., 2007). The lack of good record keeping of clinical and subclinical

mastitis and the lack of regular diagnostics make it very difficult for the herd health advisor to identify the true cause of disease, to identify associated risk factors, to monitor treatment outcomes and to evaluate management changes on the farm. The results in *Chapter 5.3*, for example, clearly demonstrate that the most effective measures to prevent IMI in early lactating heifers also depend on the (subgroup of) pathogens that (is) are involved.

Treatment of clinical mastitis

Since herds having a higher prevalence/incidence of (clinical) mastitis are using more antimicrobials (Stevens et al., 2016), they are all at a higher risk of delivering milk containing antimicrobial residues (Ruegg and Tabone, 2000). In our questionnaire, the average duration of treatment of any CM case was around 3 to 4 days. The average withdrawal time of all registered intramammary antimicrobials in 2015 in Belgium was 5 days. Taken into account the average duration of treatment and withdrawal time, the practical consequences on a 100-cow herd is that there is a risk of delivering milk with residues almost every day of the year, if the IRCM is higher than 3% per month. In our study 53.2% of the herds had a EICM higher than 3%. Logically, this results in extra stress for the producers, since penalties for residues are high and because of the extra labour that is assocated with it (e.g. collecting wasted milk, treatment, …). Labour is more and more limiting on modern dairy farms, also in Flanders, and will thus most probably affect the financial result of the herd. We should, however, mention that in Belgium every delivery of milk is tested for the presence of residues and only 0.03% of all deliveries were found positive in 2014 (Annual Report, MCC Vlaanderen, 2014). Nevertheless, in the light of prudent use of antimicrobials, farms need to focus more on prevention of CM. This is a great opportunity for herd health advisors such as the herd veterinarian. The demand for formulating standard operating procedures (SOP) will also increase in the near future since labour will be more limiting as herd size increases.

In that respect it is surprising that actually 74.4% of the producers we consulted is not using a treatment protocol for CM (*Chapter 3.1*, unpublished data). There was also a large variation in duration of treatment (from 1 to >5 days) (Figure 1) and in the way antimicrobials were administered (intramammary, parenterally or both) (Figure 2). On 46% of the farms treatment of CM was always done by the farmer, on 42% of the farms this was always done by the veterinarian, and on 12% of the herds this was only done by a veterinarian in specific cases (e.g. very severe mastitis cases). The lack of SOP is alarming since antimicrobial overuse and underdosing in food animal production has the potential to further increase the prevalence of acquired antimicrobial resistance, eventually threathening human health. The latter issue stresses the importance of avoiding residues in milk and striving for a targeted and more prudent use of antimicrobials in the dairy industry. The variation in treatment regimes of

CM on Flemish dairy farms is surprising. Producers seem to treat CM cases empirically as almost every possible treatment strategy is applied. On 66% of the herds, the off-label use of so-called “self-prepared udder infusions” with antimicrobials is actually still a regular treatment for CM. Also, when culturing of CM cases was performed, in almost 85% of the herds, antimicrobial susceptibility testing was not considered. Veterinarians should try to implement evidence-based treatment protocols on their clients’ farms. This, combined with good record keeping and susceptibility testing, should help to justify the use of antimicrobials on dairy herds and to reveal possible risk factors for IMI. In Belgium, veterinarians make a profit by delivering drugs to their clients, including antimicrobials. This conflict of interest potentially threatens their business model, yet should not prohibit vets of playing a key role in treatment decisions of dairy farmers as they are considered as one of the most positive references among dairy advisors and their advice is seen as trustworthy (Swinkels et al., 2015).

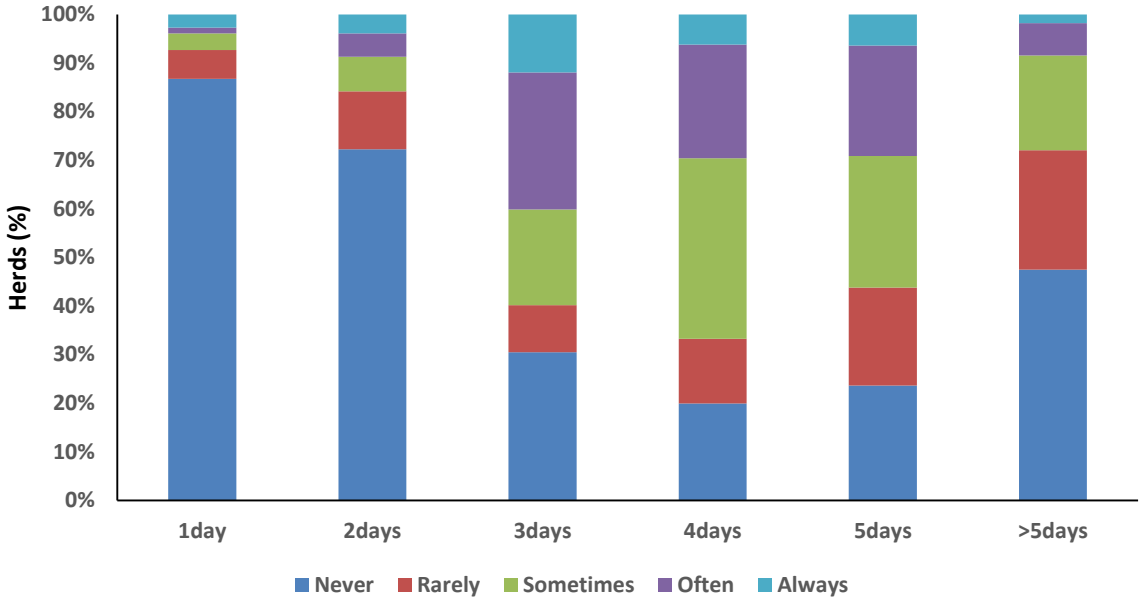

Figure 1. Distribution of Flemish dairy farms (n = 242) treating mild clinical mastitis cases in 2009 stratified by the duration of treatment. Only a minority of the herds treated mild clinical mastitis cases less than 2 days or more than 5 days. Nevertheless, the treatment duration of mild clinical mastitis cases strongly varied between and even within herds (Unpublished results from *Chapter 3.1*).

The strong variation in treatment decisions between and within farms (Figures 1 and 2) suggest that farmers feel insecure and/or have different opinions on how to treat mastitis (*Chapter 3.1)* which corresponds well with the findings of Jansen and Lam (2012). In our questionnaire 72.5% of the farmers gave the highest priority to bacteriological cure of the treated quarter rather than delivering the milk as soon as possible after treatment. Swinkels et al. (2015) also reported that none of the farmers in their study expressed concern about the

increased costs of extended treatment due to more wasted milk and higher antibiotic use. Given the large variation in treatment duration and the route of administration within herds in our study, it seems farmers (and their veterinarians) feel not confident (enough) with the guidelines given by the pharmaceutical companies. The latter typically try to bring mastitis products with a short treatment duration and with short withdrawal periods to the market, typically as a marketing strategy which, however, does not seem to based on the actual needs of the farmers. The resulting lack of confidence might pave the way for inappropriate use of antimicrobials such as extended treatments, overdosing and empirical treatments (e.g. so-called 'self-prepared udder infusions'). Pharmaceutical companies should be aware of these discrepancies and should not only focus on short treatment duration with short withdrawal periods. Also, differences between the results obtained in the registration studies and those observed in the field might be at least partly explained by the fact that most of the challenge models do not include recently calved cows, do not necessarily utilize bacterial strains that are as virulent as some of the wild types, and are generally not administered when other possible immune stressors (such as metabolic disorders or heat) are present (Erskine et al., 2003). Although difficult to solve, we advise pharmaceutical companies to consider also those issues.

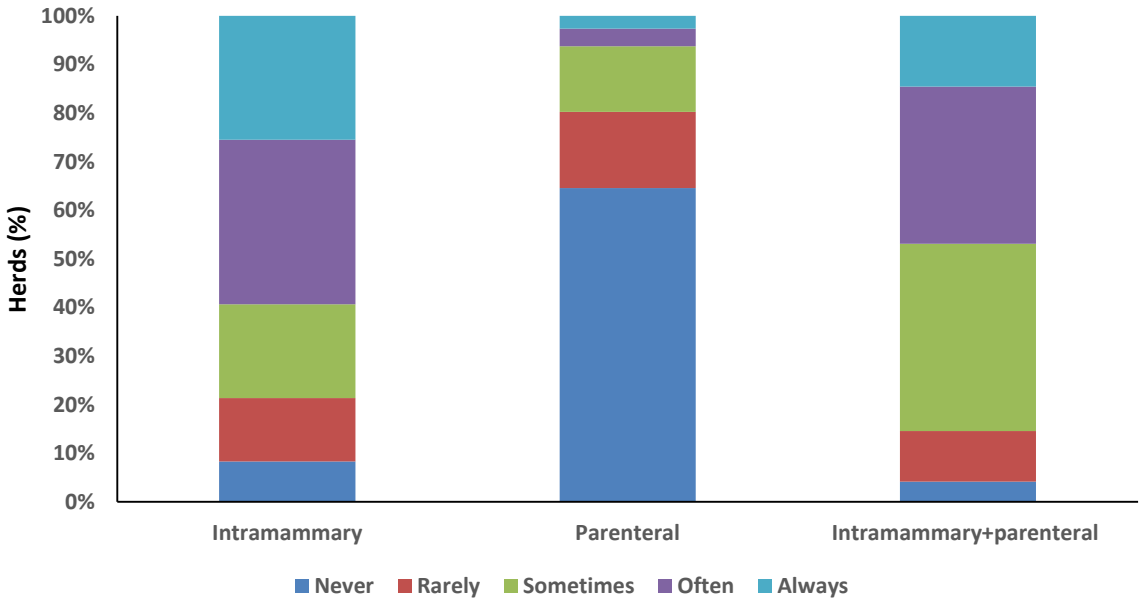

Figure 2. Distribution of Flemish dairy farms treating mild clinical mastitis cases in 2009 stratified by the route of administration. Only a minority of farms treated the mild clinical mastitis cases only systemically though the decision to treat mild clinical mastitis cases only intramammary or combinated with a parenteral treatment appeared to strongly vary between and even within herds (Unpublished results from *Chapter 3.1*).

Reproduction and culling management

Fertility is perceived by farmers as the most important topic included in VHHM programs (Derks et al., 2014) whereas advice on udder health is mostly given only when problems arise (Derks et al., 2014). Our findings (*Chapter 3.1*) suggest that the probability to have good udder health is highest in herds that monitor other aspects of cows' health besides udder health. This is not that surprising as cows with optimal reproductive performances have shorter calving intervals and are most probably again dried off earlier, increasing their chance to cure of existing IMI, in this way reducing their impact on the BMSCC and the risk of CM (Van den Borne et al. 2011). Also, farmers that join VHHM programs probably have better records on the estimated calving date, leading to a smaller variation in dry period length between cows, which consequently makes the total dry period easier to manage. Another explanation might be found in the fact that a better fertility at the herd-level offers the farmer opportunities for a more targeted culling policy of chronically infected cows, improving general udder health. Also, veterinarians might have advised farmers to get rid of sick or problematic cows where farmers would have otherwise decided differently, as was recently shown by Derks et al. (2014). Following VHHM programs are a great opportunity for farmers to improve udder health and milk quality parameters on their herd.

Milk Quality

Premium system

Payment schemes are an important incentive in controlling all bulk milk quality parameters (Veerkamp et al., 1998). Premium policies motivate farmers to produce high quality milk without providing a potential disruption to the milk supply. However, in *Chapter 4.2* we revealed that only 52.9% of the herds achieved their milk quality premium for the whole year in 2009. This was very surprising since the tresholds for the legal standards and for achieving the premium are close together. But since the premiums in Flanders only represent 3% of the milk price, farmers are probably not stimulated enough to improve the udder health status and milk quality of their herd. On the other hand, Valeeva et al. (2007) showed that non-monetary factors relating to the internal esteem, such as job satisfaction, were equally motivating as factors affecting farm economic performance. However, these authors also suggested that farmers are expected to be more motivated by a price decrease for milk with a greater BMSCC, than by a price increase for milk with a lower BMSCC. A three-stage payment system could possibly help Flemish farmers to improve milk quality: a base price could be paid for herds with

a BMSCC between 200,000 and 300,000 cell/mL. A penalty could be given to herds with a BMSCC higher than 300,000 cells/mL and a premium could be given to farms achieving low BMSCC (< 200,000 cells/mL). This strategy could also be used for the other milk quality parameters.

Interestingly, CC was the main reason for not achieving the milk quality premiums in Flanders. In our opinion, milk buyers should rethink the implementation of CC in their premium system. The use of CC results in a lot of frustration for a number of reasons (*Chapter 4.2*). Additionaly, only a small proportion of the variation of CC could be explained by manageable practices different from those related to milking and equipment hygiene (*Chapter 4.1*). We hypothesize that milk quality will improve when the BMSCC threshold is lowered and CC is excluded from the premium system. Herds with a low BMSCC are more likely to also have low CC, yet managing BMSCC is easier than managing CC on a daily base. If milk buyers still want to keep CC in the premium system, then first of all, more monthly measurements of CC are necessary. Besides that, more research has to be done on risk factors explaining the variation in CC, making it feasible in the future for farmers and advisors to implement a worthwhile strategy to keep CC values low .

We propose to change the payment system into a 3-scale system. Since CC has an added value for the milk buyers, we still prefer to measure this but to exclude the CC out of the MQP system. Instead, lowering the BMSCC threshold in a new premium system would probably also select for herds having a low CC. On the other hand, there is still room for improvement in the number of herds that achieve the premium in the current system.This latter challenge is an opportunity for farmers to improve the financial result on their farms in times with volatile milk prices.

Automated milking systems

Automatic milking is one step in a series of steps that can be taken to automate dairy production. Because of high labor costs, automatic milking is becoming more common, especially in Northwestern Europe (de Koning, 2010). The results from *Chapters 3.1* and *4.1*, along with the results of other studies (reviewed by Hovinen and Pyörälä, 2011) and personal experiences however stress the clear need for a pro-active mastitis prevention and control and milk quality plan on farms that shift from conventional to automatic milking.

Although automatic milking has some undeniable advantages over conventional milking (Edmondson, 2012), manufacturers of AMS and AMS farmers should be (made) more aware of some limitations inherent to automatic milking nowadays. An often poor cleaning of the teats before milking and an inferior detection of subclinical and clinical mastitis are some of the major challenges of automatic milking. Indeed, different studies have shown that teat cleaning in AMS is less effective than manual cleaning (Knappstein et al., 2004; Bade et al., 2008).

Equiping the automatic cleaning devices with sensors that can distinguish dirty from clean teats before cleaning, that check whether or not a teat is in the cleaning device during teat preparation or that evaluate the effectiviness of the cleaning procedure before the teat cups are attached would be very helpful in narrowing the gap in udder health and milk quality between automatic and conventional milking farms. A Finnish research group attributed the increase in BMSCC of herds equipped with an AMS to reduced separation of mastitic milk (Hovinen et al., 2009). Indeed, current AMS are yet not able to precisely distinguish abnormal milk from normal milk and to automatically separate abnormal milk, despite the fact that the European Union Legislation [Regulation (EC) No 853/2004] states that milk for human consumption has to be originate from animals free from signs of CM. In fact, in none of the numerous studies that have been performed, the ISO/FDIS 20966 (ISO, 2007) limit of 80% sensitivity with 99% specificity to detect clinical mastitis were met (Rutten et al., 2013).

As aforementioned, labor reduction and more flexible labor time appear to be the most important reasons for shifting from conventional to automatic milking (Matthijs, 2004). Personal experience learns that AMS farmers typically have more confidence in sensor technology than other farmers and will thus most probably not compensate for the inferior detection of CM cases by the AMS by a visual control. Manufacturers of AMS should thus aim to further improve the detection technologies for clinical and subclinical mastitis. In the meantime, they should take into account the fact that the reliability of alerts affects the motivation of the farmer to react (Steeneveld et al., 2010).

The strategic choice to shift from conventional to automatic milking needs to go hand in hand with an adapted daily management as it is clear that along with the shift, other factors including cow movement and activity, feeding systems, transmission routes, and detection methods for disease are changing as well, offering ample opportunity for those advising the farmer. Worthwhile to mention is that herds with a good udder health before the introduction of an AMS are more likely to have a good udder health after the change. The latter might offer opportunities for pro-active and knowledgeable vets as they are best placed to work-out a farmer-tailored mastitis eradication program prior to the shift including the detection of high SCC cows, screening for (contagious) major pathogens, and appropriate decision-making for each individual cow and to further monitor the udder health and milk quality after the shift.

Better udder health and milk quality increases profitability

Huijps et al. (2008) calculated the economic loss of a CM case to be € 210 on average, varying from € 164 to € 235 depending on the month of lactation when it occurrs. The economic losses caused by subclinical mastitis per cow present on a farm, with an average production of 8,500 kg/cow, are depending on the BMSCC (Huijps et al., 2008). For example, the losses per infected cow were €72 for a BMSCC between 100,000 cells/mL and 250,000 cells/mL and €94 for a BMSCC between 250,000 cells/mL and 400,000 cells/mL. As shown in *Chapter 3.1*, only 52.9% of the herds in our studies achieved their milk quality premium for the whole year in 2009. In Flanders, milk processors pay incentives from €1 to up €1.25 per 100 L of milk. It is likely that veterinarians are not always aware whether premiums have been achieved on their clients' farms. Even when premiums account for only 3% of the milk price in Flanders, they have a certain impact on the financial result of the farm. So, monitoring achievement of MQP is an opportunity for herd health advisors striving for better milk quality on their client's farm.

We simulated the possible losses and gains in € per cow per year in different udder health situations and whether the milk quality premium has been achieved for a whole year (green) or not (red) in a straightforward manner (Figure 3). Calculations were based on a hypothetical 100-cow herd, with an annual milk production of 8,500 kg per cow, and economic losses of mastitis were based on the abovementioned estimations proposed by Huijps et al. (2008).

By looking at Figure 3, one could calculate the gains or losses between herds of the same size with different udder health statuses. For example, if a producer with a 100-cow herd is milking an annual milk production of 850,000 kg of milk, has a BMSCC between 100,000 cells/mL and 200,000 cells/mL, an IRCM of 1,5% per month, and achieved the milk quality premium the whole year, he/she wil gain €360. In a worst case scenario (same herd does not achieve any premium, has a BMSCC between 250,000 cells/mL and 400,000 cells/mL and an IRCM of 5% per month), the herd will lose €22,000 during that exact same year. This difference is a distinctive percentage of the total amount of what could be gained by the total year production. For example, when assuming a milk price of €30 per 100 L, the latter difference determines 8.5% of the total income that could be gained through milk production on that farm. With decreasing milk prices, this percentage will further increase, making it even more profitable to focus on the udder health and milk quality on dairy farms. As shown in *Chapter 4.2*, herds with EICM > 3% per month and herds with an AMS are less likely to achieve their MQP the whole year. In the same chapter, we reported that herds which achieved the MQP the whole year, had a lower BMSCC, so hypothetically herds with a high BMSCC will probably more likely not

achieve MQP the whole year either. Also, herds with an AMS are more likely to have higher BC and CC values (*Chapter 4.1*) and to have a high BMSCC combined with a low CM (*Chapter 3.1*). The latter calculations clearly highlight the financial interest of striving for a better udder health and milk quality. Since veterinarians are seen by many farmers as the most important advisor on udder health (Jansen et al., 2010), veterinarians should grab this opportunity by integrating their recommendations and advice to improve udder health within the framework of VHHM.

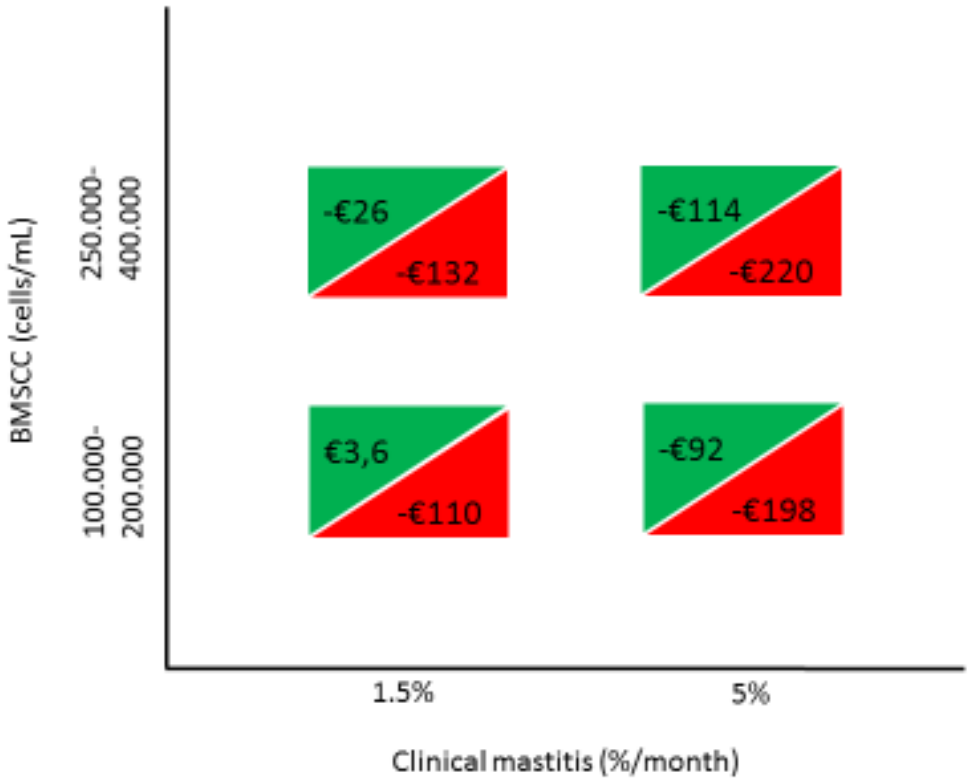

Figure 3. Losses and gains in € per cow per year under different udder health situations (average BMSCC and average monthly CM % per year) and stratified by achieving the milk quality premium for a whole year (green) or not (red). Simulations are based on a hypothetical 100-cow herd, with an annual milk production per cow of 8500 kg.

Our study on achieving premiums (*Chapter 4.2*) showed that a surprisingly low percentage of farmers achieved the premium the whole year. This leads to lost revenues in those herds. It would be interesting to calculate the exact impact of achieving milk quality premiums on the financial result of the farm, taking into account the cost of implementing management changes with a known impact (e.g. supplementation with dry minerals, application of premilking teat disinfection, lowering the incidence of CM and milking with an AMS). On the other hand, specific risk factor studies are warranted on AMS herds, since those herds are more likely to struggle with milk quality udder health issues compared to conventional milking systems.

Antimicrobial resistance and indirect effects after prepartum antimicrobial treatment of heifers

In Flanders, only a small proportion of the dairy producers (3.2%) is treating heifers prior to calving with antimicrobials (*Chapter 3.1*). In *Chapter 5.1*, we showed that systemic use of penethamate hydriodide before calving resulted in penicillin G levels in mammary tissue and secretion substantially higher than the MIC_{90} of pathogens associated with heifer mastitis. Treatment did not increase the likelihood of penicillin resistance in staphylococci (of which a majority was CNS) isolated from milk samples at calving (*Chapter 5.2*). However, selection for and spread of antimicrobial resistance takes time, making it difficult to conclude on this issue based on our data. But, when comparing the proportion of resistant staphylococci in the monitoring heifers to those from the control and treated heifers, an indirect treatment effect was suggested in the control heifers (unpublished data, Figure 4). Before the actual treatment trial started, herds were first monitored by sampling the first 8 heifers (the so-called monitoring heifers) per herd that calved. Staphylococci isolated in the monitoring period were less likely (13.7%) to be resistant to penicillin compared to the staphylococci isolated from the control (28.6%) and treated (25.6%) heifers. Strikingly, a similar finding was seen when comparing the proportion of infected quarters with any pathogens in the monitoring group (29.7%), with the prevalence of IMI found in the control (25.4%) and treated (17.9%) group, suggesting an indirect treatment effect present in the control heifers. We are very cautious in drawing conclusion based on these data, yet the findings are at least surprising and suggestive of indirect and unexpected effects that need more study. Still, we specifically avoided the statistical comparison between the data originating from the monitoring heifers and the treated and control heifers because a seasonal effect could not be excluded as data from the monitoring heifers were collected prior to those of the heifers included in the treatment trial.

Anyway, without over-interpreting the data and in the light of antimicrobial resistance, the use of short-acting antimicrobials (intramammary and parenteral use) with a narrow spectrum should be preferred in those herds that suffer from a true heifer mastitis problem [herd suffering from heifer mastitis if > 15% of heifers have CM around calving and/or if > 15% of all heifers have a first test-day SCC (measured between 10 and 35 DIM) > 150,000 cells/mL, De Vliegher et al., 2012] and that are hence likely to profit from treatment. In that respect, prepartum treatment of end-term heifers, when heifers are not producing milk, would only be defensible if it would prevent additional treatments during lactation. At that time, milk is being produced as a marketable product and treatment would reduce welfare issues and the chance of finding residues caused by CM and the required treatment.

In our prepartum treatment trial with penethamate hydriodide (*Chapter 5.2*), all 6 cases of CM in early lactation occurred in the treatment group. This might be due to chance because of the small numbers. Still, one could speculate that antimicrobial treatment disturbed the microflora in the udder (Oikonomou et al., 2012), making quarters of treated animals more susceptible to new IMI. In that respect, it is worthwile to mention the negative association between pre-existing CNS IMI and the incidence of CM cases throughout first lactation (Matthews et al., 1991; Lam et al., 1997; Piepers et al., 2010). Those findings support our abovementioned hypothesis: certain protective bacteria, such as CNS part of the milk microbiome, could be killed using penethamate hydriodide, or whatever antimicrobial treatment, making quarters less resistant.

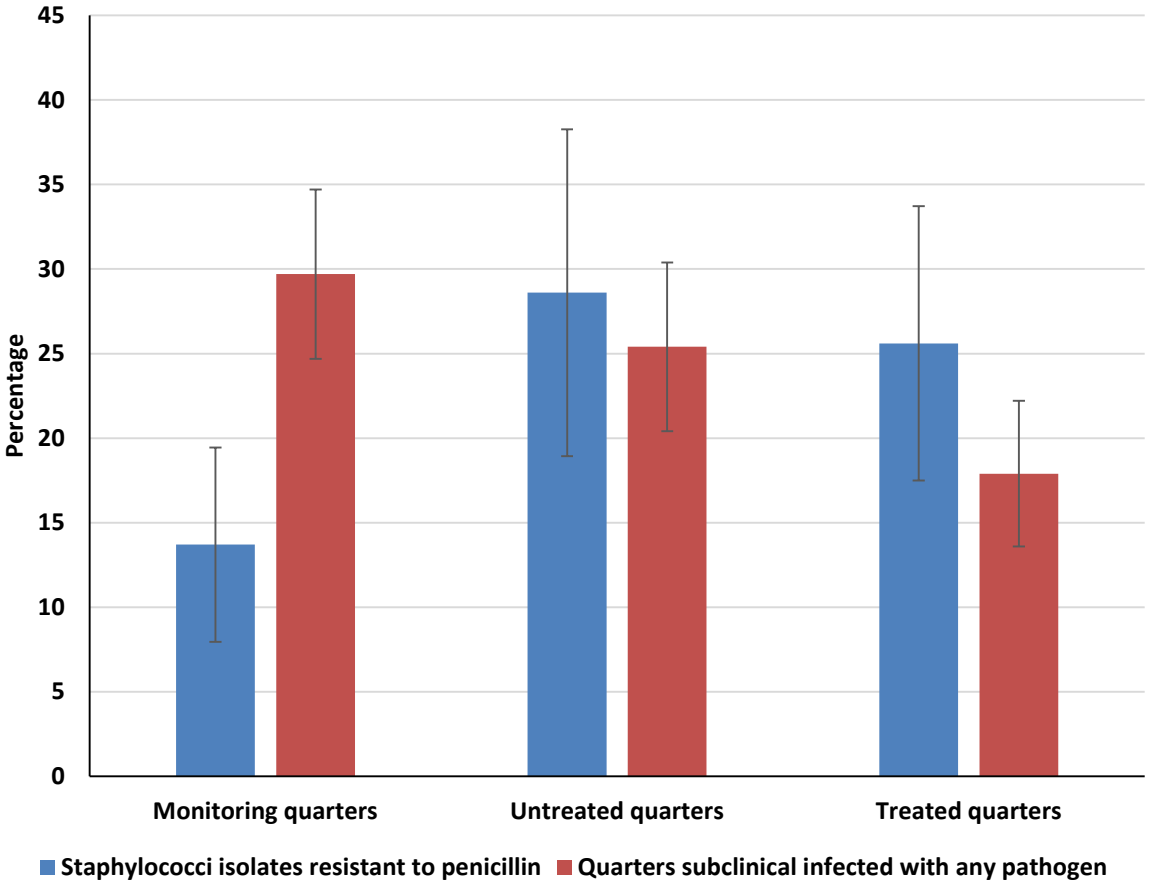

Figure 4. Percentage of isolated Staphylocci resistant to penicillin (blue) in the monitoring quarters (13.7%), the treated quarters (25.6%), and the untreated quarters (28.6%), and percentage of IMI with any pathogen (red) in the monitoring quarters (29.7%), the treated quarters (17.9%), and the untreated quarters (25.4%)(unpublished results from *Chapter 5.2*), with 95% confidence intervals.

When looking at the unconditional associations, as well as at the final models, the risk factors for IMI that were identified in treated heifers were others than those in untreated heifers, independently from the pathogen group that was considered (*Chapter 5.3*). It looks as if prepartum treatment not only changed the likelihood of infection (*Chapter 5.1*) but also the factors associated with infection. This suggests that in herds where prepartum treatment is implemented, prevention should focus on other aspects than in herds where no such programs are in place, although this needs to be substantiated in future research. The latter reasoning might also apply to blanket dry cow therapy, a mastitis control measure that has been advised for more than 40 years (Neave et al., 1969). However, given the recent concerns related to emergence of antimicrobial resistance and the preventive use of antimicrobials, selective rather than blanket dry cow therapy is more warranted. Risk factor studies that have been performed during the last decades mainly enrolled herds where blanket dry cow therapy was applied, making it likely that those factors will change on future herds where no or selective dry cow therapy is used. So, further risk factors studies on those herds will be necessary making a reduced use of antimicrobials possible.

Since farmers are often more eager to treat animals than to improve their herd (health) management (McDougall et al., 2009), herd health advisors need to be cautious in advising antimicrobial treatments, even when this would be the least expensive measure. Society expects decisions that are not (only) driven by economic aspects, but by human health concerns and animal welfare issues. Excessive use of antimicrobials should be avoided at all time. Farmers and veterinarians have a joint responsibility to ensure the correct and appriopiate use of antimicrobials with respect to the society.

Challenges and opportunities for herd health advisors

New developments in the dairy industry (i.e. abolishing milk quotas, global pressure to reduce antimicrobial usage, low milk prices) put extra demands on the udder health advisor. This thesis demonstrates that ample opportunities exist for veterinarians as udder health advisors though some challenges still need to be tackled (Figure 5). Nowadays, most veterinary practices already offer some kind of VHHM program to their clients, during which they monitor the herd health status and provide (preventive and evidence-based) advice (Derks et al., 2014). The major objective of VHHM should be to support the farmer in reaching his targets of farm performance (Noordhuizen, 2001; Noordhuizen and Wentink, 2001; de Kruif and Opsomer, 2004), yet in concordance to the expectations of the society. Several studies confirmed that mastitis-related information of veterinarians is generally considered trustworthy

and valuable (Raymond et al., 2006; Jansen et al., 2008; Swinkels et al., 2015), yet this is not always seen in the performances of the herds. Veterinary herd health programs should thus include udder health and milk quality as a specific item, all the more since our data in *Chapter 3.1* have shown that both the udder health and milk quality on the Flemish dairy herds can still improve. Still, to be effective and succesfull, the veterinary knowledge needs to be applied. Convincing farmers to effectively apply their knowledge into practice is still one of the major challenges veterinarians should solve. Veterinary herd health advisors should be aware that the farmer's decision-making and behaviour depends on his mindset which is a combination of what he wants, knows, believes and perceives (Lam et al., 2011).

Motivating farmers to take action is thus not just a matter of sharing technical knowledge but requires effective communication skills in order to get a better understanding of the farmer's mindset and the underlying explanations for a certain behaviour (e.g. social pressure). Possessing strong communication, motivation and people skills is thus a prerequisite for being succesfull as a herd health advisor.

Figures such as the simulated lost revenues due to a suboptimal udder health and the every-day risk for antimicrobial residues in the bulk tank on a 100-cow dairy herd with an EICM > 3% might be helpful in motivating the farmer as well. A lack of accurate data might be a potential bottleneck in setting-up an evidence-based and farmer-tailored VHHM program as only a minority of the Flemish farmers appears to accurately record their CM cases and only 40% of the farmers participate in a 4- to 6-weekly DHI-program. Explaining what can be learnt from those records, why it is important to know which bacteria are causing mastitis on their farm and to which antimicrobials the mastitis causing bacteria are resistant might be helpful in motivating the farmer to keep records and collect milk samples for bacteriological culturing and antimicrobial sensitivity testing in the future. Our data clearly suggest that farmers have different opinions on how to treat mastitic cows and need support in their treatment-decisions. Still, having an evidence-based treatment protocol is a necessary and useful tool on modern dairy herds. Veterinarians are among the herd health advisors undoubtely the best placed to formulate such a treatment protocol, and can by doing so clearly distinguish themselves from other herd health advisors and increase their credibility. Even so, veterinarians should also deliver value for money with those services. Establishing farmer-tailored mastitis screening, eradication and monitoring programs on farms that shift from conventional to automatic milking prior to startup and beyond seems to be a gap in a growing market that pro-active and knowledgeable vets can easily fill in. Besides the regular 4- to 6-weekly monitoring based on the available SCC and CM data, a monthly monitoring of the bulk tank milk might be recommended in particular on those farms that purchase replacement animals or on the ones

that are recovering from an outbreak of *Mycoplasma bovis* mastitis. Also, next to the 4- to 6-weekly udder health monitoring, regular fertility checks might further improve udder health as do the results from Chapter 3.1 suggest but also increase their income and that of the farmer.

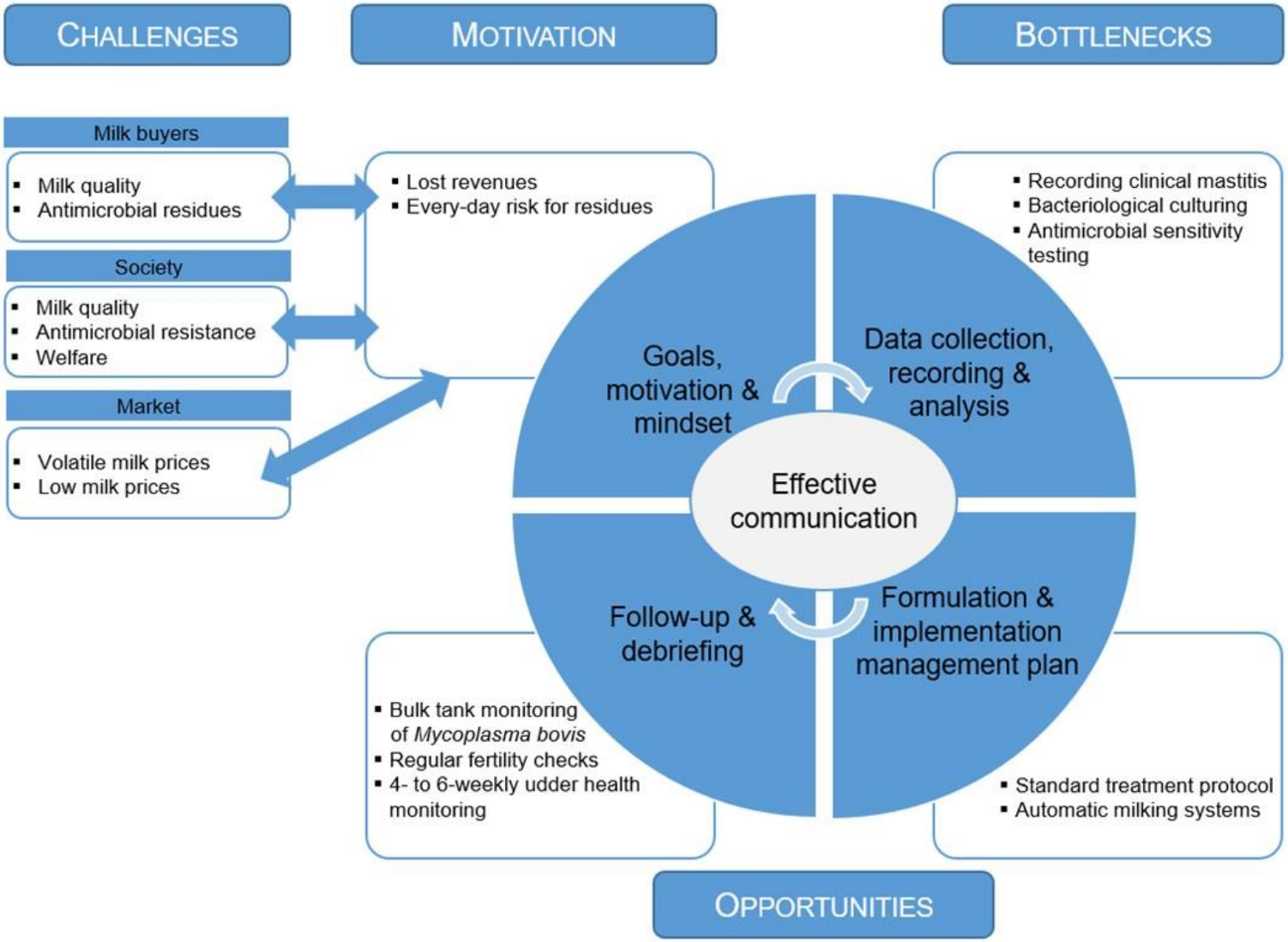

Figure 5. Challenges and opportunities for herd health adivisors in VHHM programs (adapted from Brand et al, 1996).

References

Bade, R. D., D. J. Reinemann, and P. D. Thompson. 2008. Robot versus human pre-milking teat preparation. Pages 184-185 in NMC Annual Meeting Proc., New Orleans, LA. National Mastitis Council, Madison, WI.

Barkema, H. W., J. D. Van der Ploeg, Y. H. Schukken, T. J. G. M. Lam, G. Benedictus, and A. Brand. 1999. Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. J. Dairy Sci. 82:1655-1663.

Brand, A., J. P. T. M. Noordhuizen, and Y. H. Schukken, ed. 1996. Herd health and production management in dairy practice. Wageningen Pers, Wageningen, the Netherlands.

Coffey E. M., Vinson W. E., and R.E. Pearson. 1986. Somatic cell counts and infection rates for cows of varying somatic cell count in initial test of first lactation. J. Dairy Sci. 69:552-555.

Davidson, P. M., L. A. Roth, and S. A. Gambrel-Lenarz. 2004. Coliform and other indicator bacteria. Pages 187-226 in Standard methods for the examination of dairy products. 17th ed. H. M. Weh rand J. F. Frank, ed. Am. Public Health Assoc., Asso., Washington, DC.

de Koning, C. J. A. M. 2010. Automatic milking – a common practice on dairy farms. Pages 52-67 in Proc. First North American Conference on precision dairy management, Toronto, Canada. Omnipress, Madison, WI.

de Kruif, A., and G. Opsomer. 2004. Integrated dairy herd health management as the basis for prevention. Vlaam. Diergeneeskd. Tijdschr. 73:44-52.

De Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2005a. Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. J. Dairy Sci. 88:938-947.

De Vliegher, S., H. W. Barkema, G. Opsomer, A. de Kruif, and L. Duchateau. 2005b. Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. J. Dairy Sci. 88:560-568.

De Vliegher, S., L.K. Fox, S. Piepers, S. McDougall, and H.W. Barkema. 2012. Invited review: mastitis in dairy heifers. J. Dairy Sci. 95 :1025–1040.

Derks, M., T. van Werven, H. Hogeveen, and W. D. J. Kremer. 2014. Associations between Farmer participation in Veterinary Herd Health Management Programs and Farm Performance. J. Dairy Sci. 97:1336-1347.

Edmondson, P. 2012. Mastitis control in robotic milking systems. 2012. In Practice 34:260-268.

Erskine, R. J., S. Wagner, and F. J. DeGraves. 2003. Mastitis therarpy and pharmacology. Vet. Clin. Food Anim. 19:109-138.

Guterbock, W. M., and P. E. Blackmer. 1984. Veterinary interpretation of bulk-tank milk. Vet. Clin. North Am. Large Anim. Pract. 6:257-268.

Hamann, J., and A. Zecconi. 1998. Evaluation of the electrical conductivity of milk as a mastitis indicator. Bulletin of IDF No. 334, 26pp.

Hovinen, M., M. D. Rasmussen,and S. Pyörälä. 2009. Udder health of cows changing from tie stall or free stall with conventional milking to free stall with either conventional or automatic milking. J. Dairy Sci. 92: 3696–3703.

Hovinen, M., and S. Pyörälä. 2011. Invited review: Udder health of dairy cows in automatic milking. J. Dairy Sci. 94:547-562.

Huijps, K., T. J. G. M. Lam, and H. Hogeveen. 2008. Cost of mastitis: Facts and perception. J. Dairy Res. 75:113-120.

ISO (International Organization for Standardization). 2007. Automatic milking systems – Requirements and testing. Annex C Example of methods of evaluating detection systems for milk deemed as abnormal due to blood or changes in homogeneity. ISO 20966:2007 (Geneva, Switzerland).

Jansen, J., T. J. G. M. Lam, and R. J. Renes. 2008. Mastitis control: Seize the opportunity; The role of veterinarians as effective udder health advisors. Pages 176-177 in Proc. Natl. Mastitis Counc. 47th Annu. Mtg. New Orleans, LA. Natl. Mastitis Counc., Madison, WI.

Jansen, J., C. D. M. Steuten, R. J. Renes, N. Aarts, and T. J. G. M. Lam. 2010. Debunking the myth of the hard-to-reach farmer: effective communication on udder health. J. Dairy Sci. 93:1296-1306.

Jansen, J., and T. J. G. M. Lam. 2012. The role of communication in improving udder health. Vet. Clin. North Am Food Anim. Pract. 28:363-379.

Jepsen, L., and M. D. Rasmussen. 2000. Milk quality on Danish farms with automatic milking systems. Natl. Mastitis Council Ann. Meet. 39:181-182.

Klungel, G. H., B. A. Slaghuis, and H. Hogeveen. 1998. Werk aan de robotwinkel. Melkkwaliteit bij automatisch melken verdient aandacht. Veeteelt, August, pp. 888-890.

Klungel, G. H., B. A. Slaghuis and H. Hogeveen. 2000. The effect of the introduction of automatic milking systems on milk quality. J. Dairy Sci. 83:1998-2003.

Knappstein, K., N. Roth, H.-G. Walte, J. Reichmuth, B. A. Slaghuis, R. T. Ferwerda-van Zonneveld, and A. Mooiweer. 2004. Effectiveness of automatic cleaning of udder and teats and effects of hygiene management: Report on effectiveness of cleaning procedures applied in different automatic milking systems. Deliverable D14.

Lam, T. J. G. M., Y. H. Schukken, J. H. van Vliet, F. J. Grommers, M. J. Tielen, and A. Brand. 1997. Effect of natural infection with minor pathogens on susceptibility to natural infection with major pathogens in the bovine mammary gland. Am. J. Vet. Res. 58:17-22.

Lam, T. J. G. M., J. Jansen, B. H. P. van den Borne, R. J. Renes, and H. Hogeveen. 2011. What veterinarians need to know about communication to optimize their role as advisors on udder health in dairy herds. New Zealand Vet. J., 59:8-15.

Lievaart, J. .J, W. D. J. Kremer, and H. W. Barkema. 2007. Short communication: Comparison of bulk milk, yield-corrected, and average somatic cell counts as a parameters to summarize the subclinical mastitis situation in a dairy herd. J Dairy Sci. 90:4145-4148.

Mathijs, E. 2004. Socio-economic aspects of automatic milking. Pages 46-55 in Proc. Int. Symp. Automatic Milking: A better understanding, A. Meijering, H. Hogeveen, and C. J. A. M. de Koning, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Matthews, K. R., R. J. Harmon, and B. E. Langlois.1991. Effect of naturally occurring coagulase-negative staphylococci infections on new infections by mastitis pathogens in the bovine. J. Dairy Sci. 74:1855-1859.

McDougall, S., K. I. Parker, C. Heuer, and C. W. R. Compton. 2009. A review of prevention and control of heifer mastitis via non-antibiotic strategies. Vet. Microbiol. 134:177-185.

Milk Control Center Flanders. 2014. Annual Report. Milk Control Center Flanders, Lier, Belgium.

Murphy, S. C., and K. J. Boor. 2000. Trouble-shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ. Sanit. 20:606-611.

National Mastitis Council. (2009). Recommended mastitis control program, Madison WI, USA: National Mastitis Council Inc. http://www.nmconline.org/docs/NMCchecklistInt.pdf.

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.

Noordhuizen, J. P. T. M. 2001. Changes in the veterinary management of dairy cattle: Threats or opportunities? Vet. Sci. Tomorrow April 2001:1-10.

Noordhuizen, J. P. T. M., and G. H. Wentink. 2001. Developments in veterinary herd health programmes on dairy farms: a review. Vet. Q. 23:162-169.

Oikonomou, G., V.S. Machado, C. Santisteban, Y.H. Schukken, and R.C. Bicalho. 2012. Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomic 16s rDNA. PLoS One. 7:e47671.

Peeler, E. J., M. J. Green, J. L. Fitzpatrick, K. L. Morgan, and L. E. Green. 2000. Risk Factors associated with Clinical Mastitis in Low Somatic Cell Count British Dairy Herds. J. Dairy Sci. 83:2464-2472.

Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than non-infected heifers. J. Dairy Sci. 93: 2014-2024.

Pinzon-Sanchez, C., C. Holland, and P. L. Ruegg. 2010. Post treatment outcomes of clinical mastitis on commercial dairy farms. J. Dairy Sci, E-suppl. 1:79.

Rasmussen, M. D., M. Bjerring, P. Justesen, and L. Jepsen. 2002. Milk quality on Danish farms with automatic milking systems. J. Dairy Sci. 85:2869-2878.

Raymond, M. J., R. D. Wohrle, and D. R. Call. 2006. Assessment and promotion of judicious antibiotic use on dairy farms in Washington State. J. Dairy Sci. 89:3228-3240.

Reinemann, D. J., G. A. Mein, D. R. Bray, D. Reid, and J. S. Britt. 1997. Troubleshooting high bacteria counts in farm milk. Pages 65-79 in Natl. Mastitis Counc. 36th Annual Mtg. Proc., Albuquerque, NM. Natl. Mastitis Counc. Inc., Madison, WI.

Ruegg, P. L., and T. J. Tabone. 2000. The relationship between Antibiotic Residue Violations and Somatic Cell Counts in Wisconsin Dairy Herds. J. Dairy Sci. 83:2805-2809.

Rupp, R., and D. Boichard. 2000. Relationship of early first lactation somatic cell count with risk of subsequent first clinical mastitis. Livest. Prod. Sci. 62:169-180.

Rutten, C. J., A. G. J. Velthuis, W. Steenveld, and H. Hogeveen. 2013. Invited review: Sensors to support health management on dairy farms. J. Dairy Sci. 96:1928-1952.

Schepers, J. A., and A. A. Dijkhuizen. 1991. The economics of Mastitis and Mastitis Control in Dairy Cattle: A critical analysis of estimates published since 1970. Prev. Vet. Med. 10:213-234.

Sherlock, R., H. Hogeveen, G. Mein, and M. D. Rasmusen. 2008. Performance evaluation of systems for automated monitoring of udder health: Analytical issues and guidelines. Pages 275-282 in Mastitis Control – From science to practice. T. J. G. M. Lam, ed. Proc. Int. Conf., Den Haag, the Netherlands. Wageningen Academic Publishers, Wageningen, the Netherlands.

Steeneveld, W., L. C. van der Gaag, W. Ouweltjes, H. Mollenhorst, H. Hogeveen. 2010. Discriminating between true-positive and false-positive clinical mastitis alerts from automatic milking systems. J. Dairy Sci. 93:2559-2568.

Stevens, M., S. Piepers, K. Supré, J. Dewulf, S. De Vliegher. 2016. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. J. Dairy Sci. 99:2118–2130.

Swinkels, J. M., A. Hilkens, V. Zoche-Golob, V. Krömker, M. Buddinger, J. Jansen, and T. J. G. M. Lam. 2015. Social influences on the duration of antibiotic treatment of clinical mastitis in dairy cows. J. Dairy Sci. 98:2369-2380.

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

Van den Borne, B. H. P., J. C. M. Vernooij, A. M. Lupindu, G. van Schaik, K. Frankena, T. J. G. M. Lam, and M. Nielen. 2011. Relationship between somatic cell count status and subsequent clinical mastitis in Dutch dairy cows. Prev. Vet Med. 102:265-273.

Veerkamp, R. F., A. W. Stott, W. G. Hill, and S. Brotherstone. 1998. The economic value of somatic cell count payment schemes for UK dairy cattle breeding programmes. Anim. Sci. 66:293-298.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific Incidence Rate of Clinical Mastitis in Flemish Dairy Herds, Severity, and association with Herd Hygiene. J. Dairy Sci. 97:6926-6934.

Vorst, Y. v.d., and H. Hogeveen. 2000. Pages 73-82 in Proc. Int. Symp. Robotic Milking. H. Hogeveen and A. Meijering, ed., Wageningen Pers, Lelystad, The Netherlands.

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Summary

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In response to achieve a worldwide growing demand for milk and dairy products, Flemish dairy farmers have substantially increased the average milk yield per cow as well as the average herd size. One of the diseases potentially threatening this growth and the financial profit for dairy farmers is mastitis, which can occur in two forms, a clinical and a subclinical one. Also, heifer rearing has become more and more a priority on modern dairy farms, because of the financial impact and longevity which again stresses the importance of healthy heifers at calving. Heifer mastitis can have detrimental effects on the future productive life of heifers, showing the importance of cure and prevention of IMI in heifers around calving. Besides intensification, quality and safety of dairy products have become a major concern for both producers and consumers. Next to the absence of residues, microbiological contamination of milk is an important issue in food safety as well. The aims of this thesis were to identify some new challenges related to udder health and milk quality that modern dairy farmers and their vets are currently facing or will be facing in the near future, and to learn how these can be turned into opportunities (**Chapter 2**).

Udder health

Management practices associated with the IRCM and BMSCC on Flemish dairy farms, were studied by conducting a web-based questionnaire (**Chapter 3.1**). In total, 218 farmers completed the online questionnaire that consisted of different questions concerning general management, herd health management, milking, calving and dry cow management and nutrition. Only one-third of the herds in Flanders had a low BMSCC (< 250,000 cells/mL) and a low EICM (<3%). Only a weak association between BMSCC and EICM was present. The average EICM was 4% per month, or 48% per year. However, it became clear that less than half of the farmers are recording CM cases on their farm, making the data to be cautiously interpreted. Several factors were found to be associated with good udder health, so-called environmental herds and so-called contagious herds. Veterinary herd health monitoring seemed to have added value in the prevention of udder health problems whereas in general this study gave us also more insights in how antimicrobials are applied in case of udder health issues.

Mycoplasma bovis is an emerging pathogen that can cause mastitis, with ineffective drugs treatments, culling and loss of milk production as major consequences. Early detection of cows excreting *M. bovis* bacteria is warranted, especially in expanding herds and farms that often purchase replacement animals. In **Chapter 3.2**, the prevalence of *Mycoplasma bovis* was estimated on Flemish dairy farms. Three bulk tank milk samples per herd were taken with a collection interval of two weeks. In three herds (1.5%) *M. bovis* was isolated from one of the

three consecutive bulk samples. The prevalence of *M. bovis* in Flanders was concluded to be at least 1.5% of the dairy herds. Frequent monitoring in herds at risk should be encouraged in order to detect the presence of *M. bovis* as soon as possible and to implement appropriate prevention measures when needed.

Milk quality

Associations between herd management practices and both BC and CC from 254 and 242 dairy herds in Flanders (Belgium), respectively, were studied (**Chapter 4.1**). Data were analyzed using multivariable, multilevel linear regression analysis, allowing variance components' analyses. Both BC and CC fluctuated throughout the year, although the milk quality parameters followed an opposite pattern. Bacterial count values decreased with each increase of the cleaning frequency of the cubicles (once a week, once a day, twice a day, more than twice a day) between January and March. Herds with a conventional milking parlor had substantially lower BC than herds where the cows were milked using an automatic milking system. Lower BC were observed when the milking parlor was equipped with an automatic cluster removal system, when premilking teat disinfection was applied, when the dry cows were supplemented with a mix of minerals and vitamins, and when the teats were prepared either first wet and dried or via an automatic milking system. Milking cows with a high-pipeline milking parlor set-up or with an AMS was associated with substantially higher CC values. Herds where prepartum heifers were often treated with antimicrobials before calving had a lower CC than farms where heifers were either not or only rarely treated. Most variation in BC and CC resided at the herd level rather than at the observation level, indicating that management is important in the control of both BC and CC. Still, only a small proportion of the total variance was explained by factors capturing information related to the milking, herd health, and dry cow management, which suggests that the bacteriological milk quality and in particular CC is primarily driven by other factors than the ones included in this study.

In **Chapter 4.2**, associations between herd management practices, collected through a web-based questionnaire from 242 dairy herds in Flanders (Belgium), and achieving MQP were studied. Monthly geometric means for BMSCC, BC and CC were available, as well as monthly test results of freezing point, residues and filtration. Data were analyzed using binary logistic regression. Only 52.9% (n = 128) of the herds achieved their milk quality premium all 12 months in 2009. If the BMSCC threshold would have been 250 x 10^3 rather than 350 x 10^3 cells/mL in a hMQP, only 23.5% (n = 57) would have achieved their milk quality premium every month of 2009. The main reason for not achieving the milk quality premiums in 2009 in the

current system was CC, whereas this would have been BMSCC in the hMQP system. Lowering the threshold from 350 x 10^3 to 250 x 10^3 cells/mL in the latter system, would have most likely been accompanied with lower BC and CC values. The final model revealed that herds where premilking teat disinfection was applied and where dry cows received a vitamin/mineral mix were significantly more likely to achieve the MQP in 2009. On the other hand, herds with a high (> 3%) EICM and equipped with an AMS were significantly less likely to achieve the MQP.

Heifer management

Prepartum intramammary treatment with antimicrobials of end-term dairy heifers has frequently been proposed as a practice to reduce the prevalence of IMI at calving. From a safety standpoint for both animal and administrator, systemic treatment is to be preferred. The aim of the study in **Chapter 5.1** was to assess the concentration of penicillin G in mammary tissue and secretion of dry heifers following systemic administration of penethamate hydriodide. Six dairy heifers in late gestation received a single intramuscular injection of 10 g penethamate hydriodide and were sacrificed 24, 48 or 144 hours after treatment. Penicillin G concentrations were measured in mammary tissue and secretion samples using high performance liquid chromatography. Penicillin G was detected in the udder of two animals euthanized at 24 hours (mammary tissue and secretion) and at 48 hours post treatment (mammary secretion only) after administration at concentrations still close to or above MIC_{90} values reported for the pathogens associated with heifer mastitis. Antibiotic concentration shortly after administration was substantially higher, indicating a potential for systemic treatment with penethamate hydriodide to control prepartum intramammary infections in heifers without the disadvantages of local therapy. However, this therapeutic approach needed to be verified under field conditions to quantify the short and long term effects on udder health (i.e. somatic cell counts and clinical mastitis cases), and milk production.

Hence, a clinical trial was conducted on heifers from 10 commercial, well-managed dairy farms with low prevalence of heifer mastitis (**Chapter 5.2**). The aim was to assess both the short and long-term effects of a systemic prepartum therapy with penethamate hydriodide on udder health and milk production. Because it was hypothesized that some herds would benefit more from this treatment than others, specific herd-level information was collected prior to the start of the actual trial in order to screen for and/or explain potential herd-specific treatment effects. Further, the effect of treatment on antimicrobial susceptibility of Staphylococcal isolates was monitored. End-term heifers were either treated systemically (during three

consecutive days) two weeks prior to their expected calving date with penethamate hydriodide (n = 76) or remained untreated (n = 73). Systemic prepartum treatment of end-term heifers with penethamate hydriodide resulted in fewer IMI in early lactation. However, all 6 cases of CM in early lactation occurred in the treatment group [*Streptococcus uberis* (n = 1), *Corynebacterium bovis* (n = 1), *Staphylococcus aureus* (n = 1); one sample was contaminated and two samples remained culture-negative]. No long-term treatment effects (4 to 120 DIM) on milk production, udder health or culling hazard during later lactation were detected although treated heifers belonging to herds classified as having low yielding heifers out-produced the control heifers. Moreover, penicillin susceptibility of Staphylococci isolated from milk samples of either treated or control heifers did not differ. Herds with a low prevalence of heifer mastitis were not likely to benefit from prepartum systemic antimicrobial treatment of the end-term heifers.

A number of generic risk factors associated with the likelihood of IMI in fresh dairy heifers were identified before. Yet, there was a need to identify pathogen group specific factors as the impact of (groups of) pathogens on udder health and milk yield is different. The aim of the present study was to identify pathogen group-specific risk factors for IMI in heifers participating in a prepartum antimicrobial treatment trial, allowing us to test the hypothesis that different factors are of importance between treated and untreated control heifers as well (**Chapter 5.3**). Data from a clinical trial in which end-term heifers were treated systemically (over 3 consecutive days) 2 wk before calving with penethamate hydriodide (n = 76) or remained untreated (n = 73), were available (see Chapter 5.2). A number of potential risk factors at the herd, heifer and quarter level were recorded in the first 3 DIM. Quarters from untreated heifers supplemented with ≥ 4 mg selenium/day prepartum were significantly less likely to be infected with CNS whereas quarters were more likely to be infected with CNS when assistance during calving was needed. Udder edema prior to calving significantly decreased the odds of IMI with major pathogens. In treated heifers, no factors were detected that were associated with the likelihood of CNS IMI, whereas quarters from heifers were significantly more likely to be infected with major pathogens when they were housed in the calving pen >1 day, and when they had been in contact with the lactating cows prior to calving. The risk factors for IMI that were identified in treated heifers were different than those in untreated heifers, independently from the pathogen group that was considered. It looks as if prepartum treatment not only change the likelihood of IMI but also the factors that were associated with IMI. However, except for treated heifers with an IMI caused by major pathogens, only a small proportion of the variation could be explained in the final models. So, other factors than those that were studied are explaining the likelihood of IMI at calving.

Samenvatting

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Om aan de groeiende vraag naar melk en melkproducten te kunnen voldoen, hebben Vlaamse melkveehouders hun gemiddelde melkproductie per koe en het aantal koeien per bedrijf doen toenemen. Één van de ziekten die een bedreiging kan vormen voor deze groei en voor een hoger financieel inkomen is mastitis of uierontsteking. Deze ziekte kan zich in twee vormen presenteren, nameljk een klinische en een subklinische mastitis. Door de financiële impact en het belang van langleefbaarheid - wat het groot belang van gezonde vaarzen beklemtoont - wint de opfok van vaarzen meer en meer aan belang op moderne melkveebedrijven. Vaarzenmastitis kan ernstige gevolgen hebben voor het toekomstige productieve leven. Dit geeft aan hoe belangrijk het voorkomen en genezen van intramammaire infecties bij vaarzen rond de kalving is. Naast de intensivering van de melkveehouderij, zijn kwaliteit en voedselveiligheid van melkproducten een grote zorg voor zowel producenten als consumenten. Niet alleen de afwezigheid van residuen in de melk, maar ook de bacteriologische contaminatie van melk is een belangrijk aspect van de voedselveiligheid. Het doel van dit doctoraatswerk is om enkele nieuwe uitdagingen, gerelateerd aan uiergezondheid en melkkwaliteit, die melkveehouders en hun dierenartsen vandaag en in de toekomst zullen tegenkomen, te bestuderen en om te leren hoe daarmee kan worden omgegaan (**Hoofdstuk 2**).

Uiergezondheid

De incidentie van klinische mastitis en het tankmelkcelgetal op Vlaamse melkveebedrijven, samen met geassocieerde managementfactoren, werden bestudeerd met behulp van een digitale enquête (**Hoofdstuk 3.1**). Deze online enquête bestond uit verschillende onderwerpen zoals algemeen management, diergezondheid, melktechniek, afkalf-en droogstandsmanagement en voeding en werd volledig beantwoord door 218 melkveehouders. Slechts één derde van de Vlaamse melkveebedrijven heeft een laag tankmelkcelgetal (< 250.000 cellen/mL) gecombineerd met een lage geschatte incidentie van klinische mastitis (< 3%/maand). We konden een zwakke associatie aantonen tussen beide uiergezondheidsparameters. Op basis van deze studie, was de gemiddelde geschatte incidentie van klinische mastitis 4% per maand, of 48% per jaar. Het werd tevens duidelijk dat minder dan de helft van de melkveehouders klinische mastitis gevallen registreert op hun bedrijf, wat met zich mee brengt dat men deze data met enige voorzichtigheid moet interpreteren. Verschillende risicofactoren waren geassocieerd met bedrijven met een goede uiergezondheid, met zogenaamde “omgevingsgebonden bedrijven” en met zogenaamde “koegebonden bedrijven”. Diergeneeskundige bedrijfsbegeleiding leek een positief effect te

hebben ter preventie van uiergezondheidsproblemen. Deze studie gaf ons ook meer inzicht op welke manier antimicrobiële middelen worden ingezet bij uiergezondheidsproblemen.

Mycoplasma bovis is een bacterie die mastitis kan veroorzaken waarbij geen effectieve behandeling mogelijk is. Dit kan leiden tot een sterk melkproductieverlies en een verhoogd vervangingspercentage. Een vroege detectie van koeien die *Mycoplasma bovis* uitscheiden is noodzakelijk, in het bijzonder op groeiende bedrijven die vaak dieren aankopen. De prevalentie van *Mycoplasma bovis* op Vlaamse melkveebedrijven werd beschreven in **hoofdstuk 3.2**. Drie tankmelkstalen werden genomen op 201 Vlaamse melkveebedrijven, met telkens 2 weken tussentijd. Op drie bedrijven (1.5%) kon *Mycoplasma bovis* geïsoleerd worden uit telkens een van de drie tankmelkstalen. Er werd hieruit besloten dat de prevalentie van *Mycoplasma bovis* op Vlaamse melkbedrijven 1.5% was. Frequente monitoring van risicobedrijven moet worden toegejuicht om op die manier zo snel mogelijk de eventuele aanwezigheid van *Mycoplasma bovis* op te sporen en om tijdig de nodige managementmaatregelen te kunnen nemen.

Melkkwaliteit

Mogelijke associaties tussen managementmaatregelen enerzijds en het kiemgetal en het coligetal anderzijds werden op respectievelijk 254 en 242 Vlaamse melkveebedrijven bestudeerd (**Hoofdstuk 4.1**). De data werden geanalyseerd door gebruik te maken van een multivariabele, multilevel lineaire regressie-analyse, wat analyse van variantiecomponenten toelaat. Zowel het kiem- als coligetal fluctueerden tijdens het jaar. Beide parameters volgden een tegengesteld patroon. Het kiemgetal daalde bij een stijgende frequentie van het schoonmaken van de ligboxen (1x per week, 1x per dag, 2x per dag, meer dan 2x per dag) tussen januari en maart. Bedrijven met een conventioneel melksysteem hadden een substantieel lager kiemgetal dan bedrijven die melken met een automatisch melksysteem. Een lager tankmelkcelgetal werd ook opgemerkt wanneer men beschikt over automatische afname, wanneer de spenen voor het melken werden gedesinfecteerd, wanneer droogstaande koeien gesupplementeerd werden met mineralen en vitaminen, en wanneer spenen eerst nat en daarna droog werden voorbehandeld of wanneer dit automatisch gebeurde. Melkinstallaties met een hoogliggende leiding of automatische melksystemen waren geassocieerd met een substantieel hoger coligetal. Bedrijven waar regelmatig de vaarzen met antibiotica behandeld werden voor vaarzenmastitis voor het kalven, hadden een lager coligetal dan bedrijven waar dit maar zelden of niet het geval was. De variatie in kiem- en coligetal was vooral te vinden op bedrijfsniveau en niet zo zeer op het niveau van de meting, wat een indicatie is dat het bedrijfsmanagement belangrijk is voor de controle van

zowel kiem-als coligetal. Hoe dan ook kon maar een klein deel van de totale variatie verklaard worden door de informatie behaald uit de enquête (melktechniek, diergezondheid, droogstandsmanagement,…), wat suggereert dat de bacteriologische melkkwaliteit en in het bijzonder het coligetal worden gedomineerd door factoren die niet werden bestudeerd in deze studie.

In **hoofdstuk 4.2** werden mogelijke associaties tussen managementmaatregelen en het behalen van de kwaliteitspremie bestudeerd door middel van een digitale enquête gehouden bij 242 Vlaamse melkveebedrijven. We beschikten zowel over de maandelijkse geometrische gemiddelden van het tankmelkcelgetal, kiemgetal en coligetal, als over de resultaten van de vriespuntbepaling, de filtratietest en de aanwezigheid van residuen. De data werden geanalyseerd door middel van binaire logistische regressie. Slechts 52.9% (n = 128) van de bedrijven behaalde de kwaliteitspremie elke 12 maanden gedurende het jaar 2009. Wanneer de grenswaarde van het tankmelkcelgetal in plaats van 350.000 cellen/mL, op 250.000 cellen/mL zou gezet worden in een hypothetisch melkkwaliteitspremie-systeem, dan zou maar 23.5% (n = 57) bedrijven de kwaliteitspremie iedere maand behaald hebben. De belangrijkste oorzaak in het huidige systeem voor het niet behalen van de melkkwaliteitspremie in 2009 was het coligetal. In het hypothetisch kwaliteitssysteem zou dit het tankcelgetal geweest zijn. De grenswaarde verlagen van 350.000 cellen/mL naar 250.000 cellen/mL, zou hoogstwaarschijnlijk tevens gepaard gaan met een verlaging van het kiemgetal en het coligetal. Bedrijven die de spenen voor het melken desinfecteren (spray of dipmiddel) en bedrijven waar droogstaande koeien een vitaminen-en mineralenmengsel kregen, hadden meer kans op het behalen van de kwaliteitspremie gedurende het ganse jaar 2009. Anderzijds hadden bedrijven met een hoog percentage aan – geschatte – klinische mastitis per maand (> 3%) en bedrijven die gebruik maken van een automatisch melksysteem, significant minder kans op het behalen van de kwaliteitspremie gedurende het ganse jaar.

Vaarzenmanagement

Intramammaire antibioticumbehandeling van drachtige vaarzen voor de verwachte afkalfdatum, is meermaals voorgesteld als maatregel om de prevalentie van IMI's op moment van kalven te verminderen. Vanuit veiligheidsredenen voor zowel het dier als de persoon die toedient, valt systemische behandeling te verkiezen. Het doel van de studie in **hoofdstuk 5.1** was om de concentratie van penicilline G in uierweefsel en secretie van droogstaande vaarzen te bepalen na een éénmalige injectie met penethamaat hydriodide. Zes hoogdrachtige vaarzen kregen een enkele intramusculaire injectie met 10g penethamaat hydriodide en werden nadien geëuthanaseerd op respectievelijk 24, 48 en 72 uur na de behandeling. De

concentratie van penicilline G werd bepaald in het uierweefsel en het uiersecreet met behulp van hoge performantie chromatografie. Penicilline G werd gedetecteerd in het uier bij twee dieren geëuthanaseerd op 24u (uierweefsel en secreet) en op 48u (uiersecreet) na toediening, in concentraties die dichtbij tot hoger lagen dan de MIC_{90} waarden die beschreven staan voor pathogenen geassocieerd met vaarzen mastitis. De antibioticumconcentraties kort na toediening waren substantieel hoger, hetgeen een mogelijkheid biedt aan systemische behandeling met penethamaat hydriodide in de controle van IMI's bij vaarzen, zonder de nadelen van een intramammaire therapie. Deze therapeutische benadering moet echter verder onderzocht worden onder veldomstandigheden om de korte en lange termijneffecten op de uiergezondheid (somatisch celgetal, klinische mastitis) en de melkproductie te kwantificeren.

Bijgevolg werd een klinisch veldonderzoek uitgevoerd bij vaarzen op 10 commerciële, goed gemanagede melkveebedrijven met een lage prevalentie aan vaarzenmastitis (**Hoofdstuk 5.2**). Het doel van deze studie was om zowel de korte termijn als de lange termijneffecten van een systemische prepartum behandeling met penethamaat hydriodide op de uiergezondheid en de melkproductie te bestuderen. Omdat de hypothese was dat sommige bedrijven meer zouden profiteren dan andere door deze behandeling, werden specifieke bedrijfsgegevens verzameld voor de eigenlijke start van het onderzoek met de bedoeling om eventuele bedrijfsspecifieke effecten van de behandeling te kunnen verklaren. Daarnaast werd ook het effect van behandeling op het ontstaan van antimicrobiële ongevoeligheid bij *Staphylococcus* isolaten gemonitord. Hoogdrachtige vaarzen werden ofwel systemisch behandeld (gedurende 3 opeenvolgende dagen) met penethamaat hydriodide 2 weken voor de verwachte afkalfdatum (n = 76), of bleven onbehandeld (n = 73). Systemische prepartum behandeling van hoogdrachtige vaarzen met penethamaat hydriodide resulteerde in minder IMI aan het begin van de lactatie. Alle 6 gevallen van klinische mastitis in de vroege lactatie kwamen voor bij de behandelde groep [*Streptococcus uberis* (n = 1), *Corynebacterium bovis* (n = 1), *Staphylococcus aureus* (n = 1); een staal was gecontamineerd en twee stalen waren negatief op cultuur]. Er werden geen lange termijneffecten (van 4 tot 120 dagen in lactatie) gevonden op de melkproductie, de uiergezondheid of de kans op opruimen gedurende het verdere verloop van de lactatie. Wel zagen we dat behandelde vaarzen die behoorden tot bedrijven die geklassificeerd werden als 'bedrijven met laagproductieve vaarzen' meer melk gaven dan de controledieren. De gevoeligheid voor penicilline van *Staphyloccus* isolaten van zowel de behandelde vaarzen als de controlevaarzen was niet verschillend. Bedrijven met een lage prevalentie van vaarzenmastitis hadden geen voordeel van een systemische prepartum behandeling met antibiotica van hoogdrachtige vaarzen.

Verschillende risicofactoren die geassocieerd zijn met de kans op IMI bij pasgekalfde vaarzen zijn reeds bestudeerd. Aangezien de impact van pathogenen op de uiergezondheid en de melkproductie verschillend is, is er een behoefte aan pathogeen specifieke risicofactoren te identificeren. Het doel van deze studie was om deze pathogeen specifieke risicofactoren voor IMI bij vaarzen te bestuderen tijdens een veldproef met een systemische prepartum antibioticumbehandeling (**Hoofdstuk 5.3**). Dit liet ons toe om de hypothese te testen dat de risicofactoren tussen behandelde en niet-behandelde vaarzen verschillend zijn. De data waren beschikbaar uit een veldproef waarbij hoogdrachtige vaarzen ofwel systemisch behandeld werden met penethamaat hydriodide (gedurende 3 opeenvolgende dagen), 2 weken voor de verwachte afkalfdatum (n = 76), of onbehandeld (n = 73) bleven (zie **Hoofdstuk 5.2**). Verschillende potentiële risicofactoren op bedrijfs-, vaars, en kwartierniveau werden geregistreerd in de eerste 3 dagen van de lactatie. Kwartieren van onbehandelde vaarzen die gesupplementeerd werden met ≥ 4 mg selenium/dag voor het kalven hadden significant minder kans om geinfecteerd te zijn met CNS (Coagulase-negatieve Staphylococcen). De kwartieren hadden meer kans om geinfecteerd te zijn met CNS wanneer assistentie bij het kalven moest verleend worden. Uieroedeem voor de kalving deed de kans op een IMI met major pathogenen significant dalen. Bij de behandelde vaarzen werden geen risicofactoren geassocieerd met de kans op een IMI met CNS gevonden. Kwartieren van behandelde vaarzen hadden significant meer kans op een IMI met major pathogenen wanneer ze langer dan 1 dag in de afkalfstal verbleven, en wanneer ze voor het kalven contact hadden met lacterende dieren. De risicofactoren voor IMI die we identificeerden bij behandelde vaarzen waren verschillend van die van de niet behandelde vaarzen, en dit onafhankelijk van de bestudeerde groep pathogenen. Het ziet er naar uit dat de prepartum behandeling niet alleen de kans op een IMI veranderde, maar ook de factoren die hiermee geassocieerd waren. We konden weliswaar maar een klein deel van de variatie verklaren in ons finaal model, met uitzondering voor de behandelde vaarzen met een IMI veroorzaakt door major pathogenen. Bijgevolg zullen andere factoren dan die wij bestudeerd hebben, de kans op IMI op moment van kalven veel meer beïnvloeden.

Curriculum Vitae - Publications

Curriculum Vitae

Pieter Passchyn werd geboren op 1 juli 1977 in Oostende. Na het behalen van het diploma hoger secundair onderwijs aan het Sint-Jozefsinstituut te Torhout (richting Wetenschappen-Wiskunde) vatte hij in 1995 de studie Diergeneeskunde te Gent aan. Hij behaalde zijn diploma van dierenarts (optie Herkauwers) in 2001 met onderscheiding. Zijn afstudeerwerk handelde over factoren die een invloed hebben op het tankureumgehalte van melkveebedrijven (Vakgroep Voortplanting, Verloskunde en Bedrijfsdiergeneeskunde – Promotor: Prof. dr. Geert Opsomer).

Onmiddellijk na het afstuderen associëert hij zich in de praktijk van dr. Omer Gunst te Torhout. Daarna wordt Pieter mede-zaakvoerder en groeit de praktijk uit naar dierenartsenpraktijk De Toren waar zowel grote als kleine huisdieren werden behandeld. Tijdens deze periode neemt zijn interesse toe in de begeleiding van melkveebedrijven, in het bijzonder de relatie's tussen diergezondheid, productie en nutritie. Dit resulteert in 2008 in het starten van een doctoraatstudie over uiergezondheid, melkkwaliteit en behandeling van vaarzenmastitis onder begeleiding van promotoren Prof. dr. Sarne De Vliegher en Dr. Sofie Piepers.

In mei 2010 stapt Pieter uit de groepspraktijk om zich nog meer te kunnen toeleggen op de begeleiding van melkveebedrijven en daarmee wordt Milk@vice geboren. Milk@vice is een onafhankelijk adviesbedrijf voor melkveehouders en voor bedrijven die actief zijn in de melkveehouderij. Via professioneel advies en opvolging zorgt zij voor gezonde koeien, en voor een hoge en kwalitatieve melkproductie. Daarnaast treedt Pieter verschillende keren op als stagebegeleider voor studenten van de Universiteit Gent en Vives Hogeschool. Hij wordt vaak geconsulteerd door de veevoederindustrie en treed op als 'key opinion leader' voor verschillende farmaceutische bedrijven. Pieter is tevens lid van de Raad van Bestuur van de Vlaamse Dierenartsen Vereniging en effectief lid van de Vlaamse Vereniging voor Veterinaire Epidemiologie en Economie. Hij is eveneens academisch consulent binnen de vakgroep Interne Diergeneeskunde en Klinische Biologie van de Grote Huisdieren.

Pieter Passchyn is auteur en medeauteur van meerdere wetenschappelijke publicaties in nationale en internationale tijdschriften en gaf presentaties op (inter)nationale congressen en symposia.

Publications

International & National journals

H. Van Loo, S. De Vliegher, S. Piepers, **P. Passchyn**, A. de Kruif, and G. Opsomer. 2007. Mastitis bij melkvee veroorzaakt door coliformen met nadruk op *Klebsiella* spp. Vlaams Diergeneeskundig Tijdschrift 76: 272-282.

P. Passchyn, S. Piepers, E. Schmitt-Van de Leemput, C. Guidarini, and S. De Vliegher. 2010. Concentration of penicillin G in mammary tissue and secretion of end-term dairy heifers following systemic prepartum administration of penethamate hydriodide. Journal of Dairy Research 77: 33-36.

S. Piepers, H. Van Brempt, K. Supré, **P. Passchyn**, and S. De Vliegher. 2010. Implementatie van mastitispreventie en controlemaatregelen op Vlaamse melkveebedrijven. Tijdschrift voor Diergeneeskunde 135:46-51.

P. Passchyn, S. Piepers, L. De Meulemeester, F. Boyen, F. Haesebrouck, S. De Vliegher. 2012. Between-herd prevalence of *Mycoplasma bovis* in bulk milk in Flanders, Belgium. Research in Veterinary Science, 92:219-220.

S. Piepers, Y.H. Schukken, **P. Passchyn**, S. De Vliegher. 2013. The effect of intramammary infection with coagulase-negative staphylococci in early lactating heifers on milk yield throughout first lactation revisited. Journal of Dairy Science, Vol. 96, Issue 8, p5095–5105.

P. Passchyn, S. Piepers, and S. De Vliegher. 2013. Systemic prepartum treatment of end-term dairy heifers with penethamate hydriodide: effect on udder health, milk yield, and culling until 120 days in milk. Journal of Dairy Science, 96:6324-6335.

S. Piepers, P. Zrimšek, **P. Passchyn**, S. De Vliegher. 2014. Manageable Risk Factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds. Journal of Dairy Science, 97:3409-3419.

P. Passchyn, S. Piepers, and S. De Vliegher. 2014. Pathogen group-specific risk factors for intramammary infection in treated and untreated dairy heifers participating in a prepartum antimicrobial treatment trial. Journal of Dairy Science, 97:6260-6270.

P. Passchyn, S. Piepers, G. Keefe, and S. De Vliegher. 2015. Management practices associated with achieving milk quality premiums in Flemish dairy herds. Preventive Veterinary Medicine. Submitted.

P. Passchyn, S. Piepers, G. Keefe, and S. De Vliegher. 2015. Management practices associated with clinical mastitis and bulk milk somatic cell count on Flemish dairy farms. Journal of Dairy Science. In preparation.

Oral presentations

P. Passchyn, S. Piepers, and S. De Vliegher. 2008. Treatment of subclinical mastitis in the bovine : outline of a new study' – Dutch Mastitis Research Workers, 3 December 2008, Deventer, The Netherlands.

S. Piepers, K. Supré, **P. Passchyn**, and S. De Vliegher. 2009. Evolution des concentrations cellulaires après traitement des mammites subcliniques: implication sur les notions d'efficacité et de guérison. May, France. Proceedings Société Nationale des Groupements Techniques Vétérinaires, pg 961-967.

P. Passchyn, S. Piepers, and S. De Vliegher. 2009. Prepartum treatment of heifers in prevention of heifer mastitis. Proceedings of the Dutch Mastitis Research Workers, 1 December, Wageningen, The Netherlands p. 20.

P. Passchyn, S. Piepers, and S. De Vliegher. 2010. Systemic Prepartum treatment of heifers in prevention of heifer mastitis' . Mastitis Research Workers, 3-5 November, Chicago, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Systemic Prepartum treatment of heifers in prevention of heifer mastitis: first results. Mastitis Research Workers, 3-5 November, Atlanta, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Systemic Prepartum treatment of heifers in prevention of heifer mastitis: first results. Dutch Mastitis Research Workers, 30 november, Utrecht, The Netherlands.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Systemic prepartum treatment of heifers with penethamate hydriodide in prevention of heifer mastitis: effect on milk yield, somatic cell count, clinical mastitis and culling. Mastitis Research Workers, 1-3 November, Chicago, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Risk factors for IMI in heifers included in a prepartum treatment trial. Mastitis Research Workers, 1-3 November, Chicago, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Systemic prepartum treatment of heifers with penethamate hydriodide in prevention of heifer mastitis: effect on milk yield, somatic cell count, clinical mastitis and culling. Dutch Mastitis Research Workers, 30 november, Merelbeke, Belgium.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Risk factors for IMI in heifers included in a prepartum treatment trial'. Dutch Mastitis Research Workers, 30 november, Merelbeke, Belgium.

P. Passchyn, S. Piepers, and S. De Vliegher. 2012. Systemic prepartum treatment of heifers with penethamate hydriodide in prevention of heifer mastitis: effect on milk yield, somatic cell count, clinical mastitis and culling. National Mastitis Council Annual Meeting, 22-24 January, St-Pete Beach, Florida, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2012. Systemic prepartum treatment of heifers with penethamate hydriodide in prevention of heifer mastitis: effect on milk yield, somatic cell count, clinical mastitis and culling. World Buiatrics Congress, 3-7 June, Lisbon, Portugal.

P. Passchyn, S. Piepers, and S. De Vliegher. 2014. Management Practices associated with Achieving low bulk milk somatic cell count and low incidence of clinical mastitis in Flemish Dairy Herds. Mastitis Research Workers, 7-8 August, Ghent, Belgium.

P. Passchyn, S. Piepers, and S. De Vliegher. 2015. Begeleiding van rantsoenen op melkveebedrijven. European Veterinary Conference, Voorjaarsdagen, 9 April, Amsterdam, The Netherlands. (invited)

P. Passchyn, S. Piepers, and S. De Vliegher. 2015. Hoe laat je vaarzen verantwoord afkalven op 24 maanden? European Veterinary Conference, Voorjaarsdagen, 9 April, Amsterdam, The Netherlands.(invited)

Poster presentations

P. Passchyn, and D. Gevaert. 2008. Elisa antibodies to study the spread of Mycoplasma bovis in 2 Belgian herds, World Buiatrics Congress, July, Budapest.

P. Passchyn, and D. Gevaert. 2008. *Mycoplasma bovis* sanitation program in an dairy herd using Baytril®100/Max and biosecurity measures. World Buiatrics Congress, July, Budapest.

P. Passchyn, S. Piepers, and S. De Vliegher. 2010. Systemic prepartum treatment of heifers in control of heifer mastitis. International Dairy Federation Conference, March, New Zealand.

P. Passchyn, S. Piepers, and S. De Vliegher. 2010. Systemic prepartum treatment of heifers in control of heifer mastitis, International Conference on Production Diseases, June, Gent, Belgium.

P. Passchyn, S. Piepers, and S. De Vliegher. 2011. Online mastitis survey in Flanders, Belgium: first descriptive results. International Conference of Udder Health and Communication, 25-27 November, Utrecht, The Netherlands.

P. Passchyn, S. Piepers, and S. De Vliegher. 2012. Systemic prepartum treatment of heifers with penethamate hydriodide in prevention of heifer mastitis: effect on milk yield, somatic cell count, clinical mastitis and culling. National Mastitis Council Annual Meeting, 22-24 January, St-Pete Beach, Florida, United States.

P. Passchyn, S. Piepers, and S. De Vliegher. 2014. Management practices associated with achieving low bulk milk somatic cell count and low incidence of clinical mastitis in Flemish Dairy Herds. National Mastitis Council Summer Meeting, 5-6 August, Ghent, Belgium.

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Na de studies kom je zoals zo velen in de praktijk terecht en 'verwateren' ongewild sommige relaties met oud-studenten een beetje. Maar niet met allen. Tom en Kristof. We hebben fantastische tijden beleefd als student : 'poten gaan kappen' in Oentjserk, schapen verlossen bij nonkel André en tante Nouche in Frankrijk, weken intern op de buitenpraktijk waar ik tot mijn grote verbazing nog heb gesupporterd voor een niet nader te noemen voetbalploeg in de Champions League.... those were the days! Maar ik ben heel blij dat we deze lijn 15 jaar later nog altijd kunnen doortrekken. Ook al hebben we elk van ons onze tegenslagen ook al gehad. Tom, bedankt voor je steun! Regelmatig sloten we de feestjes bij jou af met wijlen Luc De Vos door de boxen. Over andere – nog meer legendarische - feestjes zal ik niet schrijven, maar we zullen er vaak – ik toch – aan herinnerd worden. Kristof, jij bent niet te volgen. Letterlijk dan. De weekends en etentjes met Tom en jij, mondden vaak uit in gewichtheffen, bankdrukken, handenstand (niet voor mij gelukkig) of een voetbalmatch met de ganse bende. Zalig gewoon! An en Liesbeth, ook jullie bedankt ! Het moet niet makkelijk zijn, om wanneer Kristof, Tom en ik samenkomen, het gesprek van de eerste minuut tot de laatste over koeien te zien lopen. Ja, ja, we vinden altijd wel een aanknopingspunt ☺! We

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Een speciale vermelding gaat uit naar alle vrienden bij KTG Houtland: in willekeurige volgorde met de kans dat ik iemand vergeet (waarvoor nu al mijn excuses): Cé en Elke, Kris en Eva, Wouter en Nathalie, Pieter VE, Pieter VS en Wendy, Dieter, Valentijn en Katrien, Didier en Joke, Joeri en Julie, Koen en Lies, Kevin en Katrien, Davy en Jill, Bert L, Bert D, Peter, Niels, … Tennis is een mooie sport en zeker als de sfeer errond fantastisch is. Voor mij een welkome afwisseling in een al te vaak te drukke agenda. Het voordeel van tennis is dat je: 1. niet goed hoeft te zijn om te kunnen spelen en 2. je het ook op oudere leeftijd nog altijd kunt spelen (althans zo lijkt het) en 3. de derde helft altijd uitloopt ☺. We zitten dus voor meerdere jaren goed en hoeven niet direct te verleggen naar biljart, vogelpik of petanque! Mooi zo!

Mijn familie dank ik ook van harte. Alle 'Passchyntjes', de 'De Goe' s , en de 'Goolaertsjes'. Het zijn er te veel om op te noemen eigenlijk ☺. Jullie aanwezigheid hier doet me echt veel deugd! Steven, je bent er niet meer, maar 'you did something to me'. Thanks mate !

Jore en Liene, mijn oogappels. Kijk, jullie naam staat in het boekje van papa! Jullie weten van niet beter, dat papa altijd 'bezig is met de koeien'. Ofwel haalde ik de landelijke geur in huis, ofwel moest ik weer voor een paar dagen weg om 'les te volgen' of 'les te geven' ofwel zat ik thuis voor mijn computer. Dat zal niet altijd leuk geweest zijn voor jullie, maar je ziet, als je maar doorzet, komen er aan zulke dingen ook een einde! Nu zal papa echt wel meer tijd voor jullie kunnen maken. Ik kijk er al naar uit om de gewone dingen te doen met jullie, een wandeling maken, meegaan naar de volleybal, in de tuin ravotten, eens samen tv kijken,…. Vergeet niet dat het niet uitmaakt wat jullie later zullen doen, zolang je maar zorgt dat je de beste wil worden in hetgeen je doet! Straks komt er nog een broertje of een zusje (en ik weet het al ☺) bij, en kunnen jullie elk grote zus zijn!

Annelies, kijk waar we nu staan! We zijn er eindelijk geraakt. Nooit gedacht, he? Ik zeg 'we' omdat jij voor een groot stuk hieraan hebt bijgedragen. Niet alleen heb jij het geduld om tabelletjes en pagina's goed te zetten in Word (en ik niet ☺), maar heb jij ontelbaar veel dingen thuis gedaan. Het huishouden, de tuin onderhouden, voor de kindjes zorgen en een full-time job….Te veel om op te noemen, maar niet te veel om je daarvoor enorm te bedanken! Super hard bedankt, schat! Voor alles! We gaan straks uitbreiden ☺ en ik kijk er zo naar uit! Ik zie je graag!

Pieter