



Dutch Matitis Research Workers

4th Annual Meeting

DMRW



**30 November 2010
Utrecht University**

4th Annual Meeting
Dutch Mastitis Research Workers

30 November 2010
Utrecht University
F.A.F.C. Went-gebouw
Room Noord-022
Sorbonnelaan 16
De Uithof, Utrecht

Utrecht, 30 November 2010

Dear Mastitis Workers,

Welcome to, already, the 4th Annual Meeting of the Dutch Mastitis Research Workers. When we started, 3 years ago, much mastitis research was just initiated. At this moment much work has been finished. In many of the recent issues of international scientific journals such as the Journal of Dairy Science, work of our groups has been published. This means also that we are at a crossroad. With less financial possibilities as we were used to, we have to see to keep up the momentum and maintain interest in mastitis research. This meeting can be such a moment. We are glad that this year, again, the Flemish mastitis researchers are joining so that our horizons are further broadened.

The idea of this meeting is to see what others are doing, to learn from each other, to discuss each other's work but also to build up a Dutch language mastitis network, to share thought and to build friendship.

We want to thank everybody for their input and wish you a very pleasant and fruitful meeting.

Claudia Kamphuis

Theo Lam

Henk Hogeveen

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Highlights UGCN

T.J.G.M. Lam

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The highlights of the research executed during the five year mastitis project of the Dutch Udder Health Centre (UGCN) will be presented. Themes of the project were 'Bacteria', 'Cow' and 'Farmer'. Within the theme 'Bacteria' the goal was to do studies in order to be able to decrease infectious pressure. Within the theme 'Cow' the goal was to do studies related to the resistance of cows against udder infections. Finally the goal of the theme 'Farmer' was to do studies on the mindset of farmers and veterinarians: what motivates them to work on udder health. In addition to this overview, some conclusions of the research committee of UGCN on future udder health research in the Netherlands will be presented.

Bacteria

In the theme 'Bacteria' diagnostics was an important issue. A pilot was done on a multi-diagnostic PCR test to differentiate mastitis pathogens. Specifically for Coagulase Negative Staphylococci (CNS), phenotypic and genotypic typing methods were compared. The effect of CNS on udder health was evaluated in depth. The effect of biofilm on cure of *Staphylococcus aureus* was evaluated, as was the effect of other factors that were related to bacteria. A prevalence study was done on the prevalence of *Mycoplasma mastitis*. Finally studies were done on the added value of bulk milk culturing in mastitis diagnostics.

Cow

In the theme 'Cow' host resistance and milking machine were main issues. Directly related to host resistance were the studies on optimizing vitamin E levels, the effect of BVD status of dairy herds, breeding values for udder health and the study on innate immunity. Dry cow management and risk factors for heifer mastitis were also related to host resistance. Related to the milking machine were the studies on automatic cluster removers, on risk factors for herds with automatic milking systems, and on the dynamic measurement of the milking machine.

Farmer

In the theme 'Farmer' motivation was a crucial word, both by economic means as well as otherwise. Within the studies on economics, the effect of penalties and bonuses was evaluated, as well as the effect of visualizing economic losses due to subclinical mastitis. Additionally, costs and benefits of mastitis management measures were evaluated. Related to economics as well as to motivation were the studies on the optimal treatment of subclinical *Staph. aureus* mastitis. The studies on motivating factors of farmers and veterinarians to work on mastitis, on different communication strategies and on the so-called 'hard-to-reach' farmers were directly related to the motivation subject. Finally a study was in done in which the practical tools developed by the Dutch Udder Health Centre were evaluated.

Future

The research committee of the Dutch Udder Health Centre recently agreed on a memorandum in which the main subjects of future research on udder health in The Netherlands were recorded. Main issues are (molecular) epidemiology, economics, automatic milking systems, communication, large dairy herds (>150 cows), host resistance and pain. More details will be presented at the meeting.

Eindmeting meerjarenplan uiergezondheid in Nederland

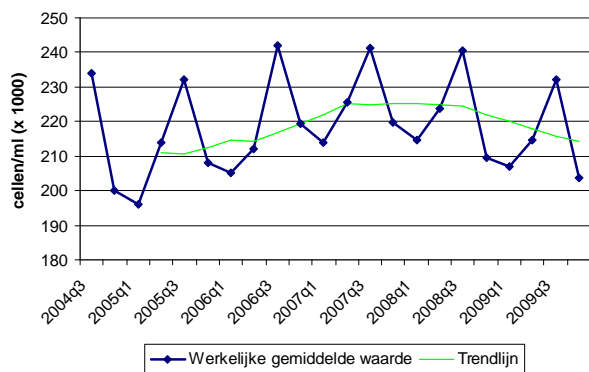
Gerdien van Schaik¹, Bart van den Borne¹, Otlis Sampimon¹, Jolanda Jansen², Theo Lam^{1,3}

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In de technische eindmeting is de mate van voorkomen van klinische mastitis en de prevalentie van dieren met een verhoogd celgetal in Nederland in 2009 vastgesteld. De resultaten zijn vergeleken met de nulmeting uit 2004/2005. Ook is in de eindmeting de invloed van de verandering in kennis, houding en gedrag van veehouders op deze mastitis kengetallen geëvalueerd.

Voor de analyse van subklinische mastitis was een dataset beschikbaar van het CRV met daarin celgetal bepalingen vanuit de melkproductieregistratie van 35.099 koeien van 266 bedrijven. In een dataset van 20.385 koeien op 175 bedrijven werden door de veehouders 4.320 gevallen van klinische mastitis opgemerkt.

Uit de berekeningen bleek dat de gemiddelde prevalentie van subklinische mastitis in 2009 20.2% was. Dit was niet significant verschillend van de prevalentie van 21.2% in 2004/2005. De incidentie van subklinische mastitis is met 0.6 gevallen gedaald, van 98.0 naar 97.4 per 100 koejaren at risk. Ook dit was geen statistisch significant verschil. In 2005 en 2006 nam het tankmelkcelgetal toe (Figuur 1). Vanaf 2007/2008, steeg het tankmelkcelgetal echter niet verder en liet het een dalende trend zien. Dit valt samen met de periode dat het UGCN



landelijk ging opereren, maar deze relatie is niet oorzakelijk aan te tonen met dit onderzoek. Het percentage dieren met een hoog celgetal liet hetzelfde beeld zien als het tankmelkcelgetal: tot 2008 een stijging, daarna een daling om ongeveer op hetzelfde niveau uit te komen als in 2004/2005. De verandering in tankmelk en koecelgetal kan slechts beperkt verklaard worden door de verandering in kennis, houding, en gedrag van veehouders; 8% gedrag, 2% kennis- en houdingfactoren.

Figuur 1. Het gemiddelde tankmelkcelgetal per kwartaal in Nederland vanaf het begin van de nulmeting (3^e kwartaal 2004) t/m het laatste kwartaal van de eindmeting (4^e kwartaal 2009) (Bron: GD Data-analyse Rundermonitor).

De incidentie van klinische mastitis is van 33.5 in de nulmeting gedaald naar 28.1 gevallen per 100 koejaren at risk in de eindmeting, een significante daling van 5.4 gevallen. De daling van klinische mastitis kan voor 21% worden verklaard door de verandering in kennis, houding en gedrag. De daling in klinische mastitis was daarnaast positief geassocieerd met de uitgangssituatie: bedrijven die een hoge klinische mastitis incidentie hadden in de nulmeting lieten een sterkere daling zien. Bij een hoge klinische mastitis incidentie is er meer ruimte om te dalen, maar de associatie met de verandering in kennis, houding en gedrag factoren geeft aan dat de mindset van veehouders ook van invloed is op de uiergezondheid. Ook externe factoren speelden mogelijk een rol. Zo hebben in de periode 2004-2009 blauwtong, sterk fluctuerende melkprijzen, en de toename van automatische melksystemen waarschijnlijk veel invloed gehad. In België is er in dezelfde periode een verslechtering van de uiergezondheid opgetreden.

Parameters for natural resistance in bovine milk: a potential role for antibodies

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Mastitis is one of the most important health problems of dairy cattle. Resistance against mastitis and many other diseases is partly based on the naturally present disease resistance capacity: the innate immune system. This research aimed to identify new immune parameters and determine their relation with the susceptibility of the individual animal for mastitis. Ideally these immune parameters can be easily determined in milk enabling routine screening of dairy cows. To potentially be an adequate marker of natural resistance, such immune parameters should first show variation among cows and repeatability within cows over time. Levels of total natural antibodies (NAb) and IgG1, IgG2, IgM and IgA binding the Pathogen Associated Molecular Patterns (PAMP) lipopolysaccharide (LPS) from *Escherichia coli* and lipoteichoic acid (LTA) and peptidoglycan (PGN) from *Staphylococcus aureus*, and the truly naive antigen keyhole limpet hemocyanin (KLH) from the sea mollusk *Megathura crenulata* were suitable to further study their relation with natural resistance of dairy cows.

In milk samples from 1515 heifers, from the Milk Genomics study, information on clinical mastitis (CM) and somatic cell count (SCC) of this first lactation was available. With these data the relation of the different NAb titers with risk for CM and high SCC later in lactation was studied. Two datasets were created. One contained data of all the heifers; the all-heifers dataset (n = 1515). The other dataset contained data from heifers that did not show CM nor had high SCC (>150,000 cells/mL) during the SCC test-days before or at the moment of NAb measurement (n = 906); the healthy-heifers dataset. For both datasets, the relation of NAb levels with the risk for CM or high SCC was studied with logistic regression. In the all-heifers dataset for nearly all NAb statistically significant odds ratios (OR) > 1 for risk for high SCC were found. In both datasets IgG1 NAb binding KLH significantly decreased the risk for high SCC (OR = 0.93 (all-heifers) and 0.86 (healthy-heifers)). Only in the healthy-heifers dataset higher total and IgG1 NAb titers binding LPS also significantly decreased the risk for high SCC (OR = 0.86 resp. 0.88). In the healthy-heifers dataset the OR of IgM and IgA binding all antigens for the risk for CM was below 1 (OR = 0.74 ó 0.85), suggesting a protective effect of these isotypes against CM, albeit not statistically significant.

Furthermore, a field study was conducted to validate if NAb titers present in milk samples taken during the first 3 months of lactation had a prognostic value for the risk for CM and high SCC (>150,000 cells/mL for heifers and >250,000 cells/mL for multiparous cows) later in that lactation. In single milk samples from 1998 cows of 60 farms the isotypes IgG1, IgM and IgA binding the 4 antigens were determined. From these (heifers and multiparous) cows information on diseases and test-day SCC of that lactation were used to create 2 datasets similar to the ones of the Milk Genomics data; an all-cows dataset and a healthy-cows dataset. Logistic regression analysis indicated IgG1 binding KLH in the healthy-cows dataset to be protective against CM (odds ratio 0.8), but not against high SCC.

Our data suggest that higher NAb titers in milk can reflect higher resistance to mastitis. Heifers differ from multiparous cows in the relation of selected NAb with resistance to mastitis, and udder history affects this relation. This research reveals the putative importance of NAb in prevention of CM and high SCC in dairy cows of different parities.

Polymorphisms in the genes *CXCR1* and *CXCR2* and the relation with udder health of dairy heifers

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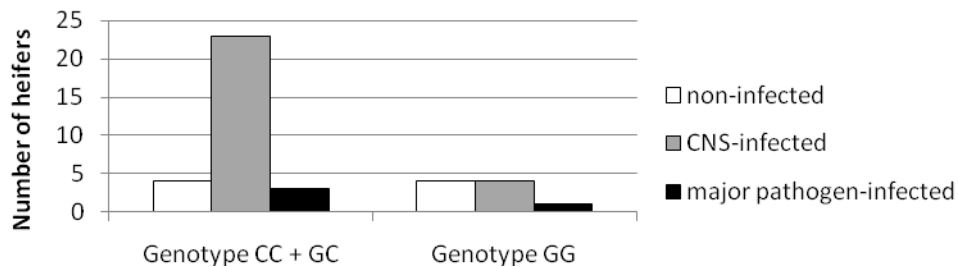
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Introduction

The prevalence and incidence of heifer mastitis differs among farms. Yet, an even larger variation is observed among heifers within the same farm under the same management, suggesting genetic predisposition for mastitis susceptibility¹. Although heifer mastitis is a multifactorial and polygenetic trait, some so called 'major genes' might exist. We opted to screen the genes *CXCR1* and *CXCR2* for polymorphisms and to analyse whether these are associated with mastitis susceptibility. The genes code for the homonymous receptors binding interleukin 8 (IL8). This chemokine plays a crucial role in the innate immunity of the mammary gland which forms the first line of defence against invading mastitis pathogens. Besides their important function, findings by other research groups on *CXCR1*^{2,3} stimulated us to investigate these genes in more detail.

Preliminary results

The entire coding region of *CXCR1* of 140 heifers (originating from 20 farms; Piepers, 2010) was sequenced and screened for polymorphisms. In total, 20 single nucleotide polymorphisms (SNPs) were found from which 15 have not been described in literature before. Heifers with the genotype GG at position 735 of coding sequence tended to have lower odds of intramammary infection (IMI) by all pathogens at calving ($P = 0.063$). However, no associations were detected between this SNP and SCC or milk production during first lactation. Heifers with genotype GG seemed to be more resistant to IMI caused by coagulase-negative staphylococci (CNS; $P = 0.055$) known to induce only moderate changes in SCC and milk production. The results suggest a pathogen-specificity in the altered mastitis susceptibility caused by the mutation.



Future research plans

First, the coding region of *CXCR2* and the non-coding regions of both genes of the 140 heifers will be screened for polymorphisms. Next, the association between the *CXCR1* and *CXCR2* genotype and IMI at calving will be further investigated. Finally, a cohort study on 30 farms will be performed to analyse the impact of the *CXCR1* and *CXCR2* genotype on the incidence of clinical and subclinical mastitis.

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2. Youngerman S.M., Saxton A.M., Oliver S.P., Pighetti G.M. (2004) Association of *CXCR2* polymorphisms with subclinical and clinical mastitis in dairy cattle. *Journal of Dairy Science* **87**, 2442-2448.
3. Leyva-Baca I., Schenkel F., Martin J., Karrow N.A. (2008). Polymorphisms in the 5' upstream region of the *CXCR1* chemokine receptor gene, and their association with somatic cell score in Holstein cattle in Canada. *Journal of Dairy Science* **91**, 407-417.

Genotypic characterization and antimicrobial susceptibility of CNS from cowsø milk and environment

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Coagulase-negative *Staphylococcus* (CNS) species are the predominant group of organisms causing intramammary infections (IMI) in dairy cows of all ages. It is hypothesized that (part of) these CNS IMI could originate from cowsø environment. This was investigated in a longitudinal study conducted on 6 Flemish dairy herds. Epidemiology of individual CNS species causing IMI was studied, as well as presence of the methicillin-resistance gene (*mecA*) and biofilm genes (*ica* and *bap*), and antimicrobial resistance of CNS from IMI and cowsø environment.

CNS isolates from milk (n = 134) and environment (n = 637) were genotyped and identified to species level using AFLP¹. The species causing IMI were *S. chromogenes*, *S. haemolyticus*, *S. epidermidis*, and *S. simulans*. *S. chromogenes* and *S. epidermidis* were rarely isolated from environment, in contrast with *S. simulans* and *S. haemolyticus*, which had a reservoir in the environment. The CNS species predominating in cowsø environment were *S. equorum*, *S. sciuri*, and *S. haemolyticus*. All isolates belonging to the 4 species causing IMI were additionally genotyped by RAPD². For *S. chromogenes* (n = 57) and *S. epidermidis* isolates (n = 29), 5 and 6 genotypes (combination of AFLP- and RAPD-type) were identified, respectively. *S. simulans* (n = 50) and *S. haemolyticus* (n = 143) were genetically more heterogeneous, showing 16 and 24 genotypes. Same genotypes were found in the environment and the milk especially for *S. haemolyticus* (9 genotypes were found in both niches).

The *mecA* gene was detected in *S. chromogenes*, *S. epidermidis*, and *S. simulans* isolates and in environmental *S. sciuri* and *S. fleurettii* isolates. Biofilm genes were rarely detected in the IMI causing species, but were common in *S. equorum* (*bap*), *S. sciuri* (*ica*), and *S. xylosus* (*bap* and *ica*). Susceptibility of 82 CNS isolates for 5 antibiotics (table 1) was tested by Etests (ABbiodisk). Only for erythromycin (14.6%) and oxacillin (11.0%), resistant strains were found. Of the 9 OX-resistant CNS, 8 possessed the *mecA* gene.

In conclusion, epidemiology varies between CNS species. *S. chromogenes* and *S. epidermidis* are likely more host-adapted, while *S. simulans* and *S. haemolyticus* are able to survive in environmental conditions and possibly act as environmental pathogens. The *mecA* gene was found both in CNS causing IMI and living in the environment. Phenotypic OX-resistance was detected in *S. sciuri* and *S. epidermidis* isolates and was associated with presence of *mecA*. A reservoir of biofilm genes was found in CNS from environment, but not from milk. Consequently, no association between biofilm formation and virulence of CNS was shown.

Table 1. Number of CNS isolates resistant (intermediately resistant) to individual antibiotics.

CNS species	no. of isolates	Antimicrobial drugs				
		CE	EF	EM	GM	OX
<i>S. chromogenes</i>	10	0 (0)	0 (0)	2 (0)	0 (0)	0 (2)
<i>S. epidermidis</i>	10	0 (0)	0 (0)	3 (0)	0 (0)	3 (0)
<i>S. simulans</i>	10	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)
<i>S. haemolyticus</i>	32	0 (0)	0 (0)	3 (0)	0 (0)	0 (0)
<i>S. sciuri</i>	10	0 (0)	0 (1)	1 (0)	0 (0)	6 (2)
<i>S. equorum</i>	10	0 (0)	0 (0)	2 (7)	0 (0)	0 (0)

¹Piessens V et al. (2010), J Microbiol Methods

²Fitzgerald JR et al. (1997), Epidemiol Infect

Outline of a new study: neutrophil viability in dairy cows: predicting potential, dynamics and associated factors

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The innate immunity plays a key role in the first line immune defense of the mammary gland. Innate immune responses of the mammary gland are mainly determined by the number and function of circulating, resident and incoming neutrophils. Looking for an alternative to reduce the risk of clinical mastitis, the role of neutrophils in the development, severity, and cure of a clinical mastitis will be explored under both field and *in vitro* conditions. In this regard, the neutrophil viability will be studied because it seems a reliable predicting parameter to determine the strength of the innate immunity of the mammary gland and the bovine in general. Additionally, factors at the herd- and cow-level that might explain the variation in blood neutrophil viability before calving and in both blood and milk neutrophil viability during lactation will be screened.

First, a longitudinal field study will be performed on 20 farms with a total of 240 animals. The predicting potential of the blood neutrophil viability will be investigated in the dry period (7 days prior to calving) for the risk of clinical mastitis within the first 14 days after calving. The study results will reveal whether or not a high neutrophil viability protects against a clinical mastitis and to what extent the severity and the duration of a clinical mastitis is associated with the blood neutrophil viability. After calving, blood and milk samples will be collected monthly throughout the first eight months of lactation to determine the dynamics of the blood and milk neutrophil viability and the association between those two parameters. Both blood¹ and milk² neutrophil viability will be evaluated using previously described flowcytometric methods.

Secondly, an *in vitro* study will be conducted in order to unravel the interaction between the most common mastitis pathogens and blood as well as milk neutrophils, respectively. A dozen bacterial species will be selected from the clinical cases in the field study, which will all be incubated with both blood and milk neutrophils isolated from eight different cows (four with a high and four with a low neutrophil viability). In this part of the study neutrophil viability^{1,2}, oxidative burst activity³ and phagocytotic capacity³ will be determined using the flowcytometer.

The aim of this research is to analyse whether or not and to what extent the neutrophil viability predicts the susceptibility of a cow to develop a clinical mastitis. This will eventually lead to a practical tool to evaluate the innate immunity on farm. Subsequently, the factors influencing the viability will be studied to establish new management strategies to improve the innate immunity.

¹ Van Oostveldt K., Vangroenweghe F., Dosogne H., and Burvenich C. (2001). Apoptosis and necrosis of blood and milk polymorphonuclear leukocytes in early and midlactating healthy cows. *Veterinary Research*, 32, 617-622.

² Piepers S., De Vlieghe S., Demeyere K., Lambrecht B., de Kruif A., Meyer E., and Opsomer G. (2009a). Technical note: flow cytometric identification of bovine milk neutrophils and simultaneous quantification of their viability. *Journal of Dairy Science*, 92, 626-631.

³ Smits E., Burvenich C., and Heyneman R. (1997). Simultaneous flow cytometric measurement of phagocytotic and oxidative burst activity of polymorphonuclear leukocytes in whole bovine blood. *Veterinary Immunology and Immunopathology*, 56, 259-269.

Systemic prepartum treatment of heifers in prevention of heifer mastitis: first results

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Prepartum intramammary treatment with antibiotics has frequently been proposed and successfully evaluated as a practice to reduce the prevalence of intramammary infections (IMI) in heifers at calving. Long-term positive effects could not be demonstrated in every study. Moreover, most studies have focused on the therapeutic effect of intramammary treatment using either dry cow or lactating cow products and only rarely on the effect of systemic treatment of heifers prior to calving. The latter approach is, however, more practical, and it is safer for the treated heifers as well as for the person who is administering the product. The objective of the study was to test whether or not systemic therapy with penethamate hydriodide of preterm heifers on 10 Flemish dairy farms is effective in enhancing the clearance of periparturient IMI, in increasing milk production, in decreasing somatic cell counts (SCC \emptyset), in reducing the incidence of clinical mastitis, and in reducing the culling rate during the first four months of lactation. The identification of herd- and heifer-level factors that may influence these processes and are associated with the presence of IMI at parturition, were secondary objectives. A randomized clinical trial was conducted: heifers were either treated 14 days prior to expected calving date with penethamate hydriodide or remained untreated. The study was designed to look for possible herd times treatment effects. A monitoring period was included in the study design prior to the actual treatment period in which only heifers were sampled at calving for culturing. Data gathered from that monitoring period is being used to explain possible herd times treatment effect by categorizing herds based on heifer culture data. Preliminary results indicate that prepartum treatment results in a higher milk production only in herds categorized as not having a high prevalence of CNS IMI.

Subclinical mastitis effects cure rate of clinical mastitis

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Introduction

Evaluation of cure rate of clinical mastitis under field circumstances is difficult, because of lack of all necessary data. Nevertheless it is important to get information about the effect of treatment based on available data in the field. Introduction of a new intra mammary injector to treat clinical mastitis was reason to design a small clinical trial under field conditions.

Study design

Five commercial dairy farms with more than 25 % cases of clinical mastitis per year were enrolled in the study. From each farm, the first 10 cows with clinical mastitis were treated with the new injector, independent the severity of mastitis, the age of the cow or if it was a new case or a repeated one.

The herdsmen were free to treat their cows like they always did, with regard to treatment length and additional parental treatment (be it antibiotics and/or anti-inflammatory drugs). They were only asked to use the new injector for intra mammary use. Further on they were asked to record clinical data and take milk samples at the start of treatment and 14 days after the start of treatment. Somatic cell count data was gathered from DHIA records. In addition, retrospective data was gathered from the 10 mastitis cases on each farm which had occurred previously to the start of the experiment. A total of 40 mastitis cases were included in the study and were compared with retrospective data of 40 cases of clinical mastitis on the same farms. Cure rate of the new mastitis injector was evaluated on three levels: clinical cure, bacteriological cure and based on somatic cell count. The SCC patterns were analysed and definition of cure was based on the guidelines of the UGCN. The performance of the new injector was compared to the retrospective data on SCC level.

Results

In all but 1 cases the farmers reported the absence of clinical mastitis signs after completion of treatment. Length of treatment was strongly effected by the absence of clinical signs.

The use of bacteriological cure seemed to be an unreliable method due to the number of contaminated samples which were taken by the farmers.

The overall SCC cure rate (new and retrospective data combined), defined as low SCC in 2 consecutive milk samplings taken at least 14 days after treatment, was 62% for both the new injector and the injectors used in the retrospective cases.

Taken the SCC history of the cow into account cure rates showed a dramatic shift.

Overall mastitis cases with a low SCC history had a cure rate of 80%, cases with a high SCC history show a cure rate of only 30%. These changes in cure rate based on the SCC level of cows before the clinical mastitis occurred showed the same trend for the cases treated with the new injector and those were treated in the retrospective data.

Conclusion

Based on this data, it can be concluded that cure rate of clinical mastitis in cows that already suffered from subclinical mastitis is very poor, independent the choice of intra mammary treatment. Treatment of chronic infections, either subclinical or clinical, seems to be a waste of the use of antibiotics. With the focus of less antibiotic use in the future, it is questioned if cows as described above should be treated with antibiotics at all. Clinical guidelines, based on results of research, should be developed to inform farmers whether to treat their animals with antibiotics or not.

The Canadian Bovine Mastitis Research Network

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Despite continuous advances in research and development, mastitis still occurs in more than 20 per cent of Canadian dairy cows with associated annual losses estimated over \$400 million. That makes it the most frequent and costly disease in the dairy industry, and an ongoing challenge for veterinarians and researchers as well. To meet this challenge, Canadian dairy producers and researchers created the Canadian Bovine Mastitis Research Network (CBMRN), a partnership between the provincial dairy boards, Dairy Farmers of Canada, Canadian Dairy Network, Natural Science and Engineering Research Council of Canada (a scientific funding organization of the Government of Canada), 9 universities and 2 research stations, and 38 researchers. The 5-year research program started in 2006 with approx. Can\$7M in funding and has leveraged \$1.3M more. Currently, it has 18 research projects with over 40 graduate students and postdoctoral students.

The Core Research Platform is the centre of CBMRN research activities. Its main components are the National Cohort of Dairy Farms, the Mastitis Laboratory Network and the Mastitis Pathogen Collection. In short, 91 dairy farms across six Canadian provinces with an average of 85 cows per farm provided data on their management and udder health practices and allowed for the collection of 137,000 milk samples and 700 DNA samples. A central database consisting of information about demographics, herd management, health, use of antimicrobials, genetics and production at the cow and the herd level was compiled. This database is cross-referenced with the approximately 17,000 bacterial isolates that make up the Mastitis Pathogen Culture Collection.

Knowledge transfer is a major focus of the CBMRN. Our portfolio now offers illustrated factsheets, videos, calculation tools and a newspaper devoted to research which has been distributed to the 13,500 dairy producers across Canada, with the assistance of provincial dairy associations. We have also produced a kit specially designed for Canadian veterinarians, based on material of, and in collaboration with the Dutch Udder Health Centre.

This year, a lot of the Network's resources were mobilized toward the continuation of our activities beyond 2011. With the help of a consulting firm, the recommendations of our partners and other organizations and the support of our members, a new vision emerged from the extensive consultation process: the sustainable production of safe, high-quality milk. This new vision encompasses 1) understanding the fundamental mechanisms involved in the persistence of pathogens in dairy herds which hinders the sustainable production of safe, high-quality milk, 2) developing new management practices, and 3) transferring new knowledge to dairy producers, veterinarians and other stakeholders. Over the next year, we will be soliciting the financial support of current and new partners alike, in addition to ensuring the continuation of public funding for the second phase of the CBMRN. At the same time, research, training and knowledge transfer activities are continuing from November 2010 to December 2012, as the CBMRN contributes the udder health focus to the Dairy Farmers of Canada-Agriculture and Agri-Food Canada Dairy Science Cluster. The projects undertaken within the framework of the Dairy Science Cluster will be in line with the new emerging vision of the Network.

Sensor measurements revealed; predicting the Gram-status of clinical mastitis causal pathogens

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Automatic milking systems produce mastitis alert lists that report cows likely to have clinical mastitis (CM). A farmer has to check these listed cows to confirm a CM case and to start an antimicrobial treatment if necessary. Currently, the decision which antimicrobial treatment to use is made without any knowledge about the pathogen that causes the CM, as the whole processing from taking a milk sample, culturing it, and determining the pathogens usually takes three days. In order to make a more informed decision about which antimicrobial to use, it would be beneficial for dairy farmers to have information about the CM causal pathogen at the same time a cow is listed on the mastitis alert list. Therefore, this study explored whether decision-tree induction was able to predict the Gram-status of CM causal pathogens using in-line sensor measurements from automatic milking systems.

Data were collected at nine Dutch dairy farms milking with automatic milking systems and included 140 bacteriological cultured CM cases with sensor measurements of electrical conductivity, colors red, green, and blue and milk yield for analyses. In total, 110 CM cases were classified as Gram-positive CM cases and 30 as Gram-negative. Stratified randomization was used to divide the data in a training set ($n = 96$) for model development, and a test set ($n = 44$) for validation. The trained decision tree used three variables to predict the Gram-status of the CM causal pathogen; two variables were based on electrical conductivity measurements, and one on measurements of the color blue. This decision tree had an accuracy of 90.6% and a kappa value of 0.76 based on data in the training set. When only those CM cases were considered with extreme high probability estimates for their Gram-status (either positive or negative), 74% of all records in the training set could be classified with a stratified accuracy of 97.1%. When validated, the decision tree performed poorly; accuracy dropped to 54.5% and the kappa value to -0.20. The stratified accuracy calculated for 75% of all records in the test set was 66.7%. Based on these results, it is concluded that sensor information from the electrical conductivity, color, and milk yield provide insufficient discriminative power to predict the Gram-status or the CM causal pathogen itself. However, the development of new or improved sensors may be used for future prediction models. Based on a study conducted by Steeneveld *et al.* (2009), somatic cell count (SCC), color and whether a cow was sick at the time of a CM infection were significant cow factors to predict the Gram-status of a CM pathogen. These three cow factors can be measured by sensors: sensors estimating the SCC on-line already exist, a sensor measuring udder skin temperature may be used as indicator of sickness, and improved color sensors that measure light transmittance rather than light reflection are available as well. Therefore, although the decision tree developed in the current study performed poorly on data from the test, it could very well be that other sensors than used in the current study are able to contribute to predict the Gram-status or CM causal pathogens.

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Unraveling the bacterial colonization diversity of teat apices of bovine udders through culture-dependent and culture-independent analysis

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Introduction

Bovine clinical and subclinical mastitis is an infection of the udder of dairy cows caused by a variety of micro-organisms resulting in financial losses due to premature culling, milk production losses, and an increased risk of recurring infections.

Aims

The micro-organisms lining the teat apices of the udder are among the most likely to enter the intramammary tissue. Determining the bacterial ecosystem of the teat apices can be of great interest, as a balance between protective and infection-causing bacteria will play an important role in health and disease. Particular interest goes to the determination of the *Staphylococcus* species diversity.

Materials and methods

Teat apex samples from cows on two different Flemish dairy herds were gathered. Estimations of the bacterial species diversity were performed through microbial growth (classical plating, incubation, and isolation of bacteria) on several selective and non-selective agar media and analysis of the isolates by (GTG)₅-PCR fingerprinting. Culture-independent analysis was performed through PCR-DGGE using universal primers (16s rRNA gene) for an estimation of the overall species diversity.

Results

Large bacterial species diversity was found on the teat apices of bovine udders, revealing a complex ecosystem of environmental bacteria, potentially infectious bacteria, and commensal skin-associated bacteria. Determining the colonization rate, being the amount of teat apices from which a respective species was isolated, revealed the dominance of certain bacterial genera and species. The *Staphylococcus* species diversity was significant; a total of 15 different species was isolated. The species could be divided into several fingerprint type (ftp) groups through (GTG)₅-PCR fingerprinting. On herd one, 40.6% of all teat apices allowed the isolation of *S. equorum*, 56.3% *S. haemolyticus* fpt I, 15.6% *S. cohnii* subsp. *cohnii*, and 12.5% *S. saprophyticus* ftp I, while on herd two *S. haemolyticus* fpt I was present on 32.5%, *S. equorum* on 5%, and *S. cohnii* subsp. *cohnii* on 7.5% of the teat apices, while *S. saprophyticus* ftp I was not isolated. The non-*Staphylococci* with the highest colonization were *Kocuria* species, divided over eight distinctly different fingerprints. *Kocuria* species are skin-associated bacteria with opportunistic pathogenic properties. For this genus also significant differences in colonization were detected between the two herds, emphasizing a distinctly different ecosystem present between farms.

Outline of a new study: coagulase-negative staphylococci: some species do cause trouble - Risk factors, persistence and sources of infection

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Coagulase-negative staphylococci (CNS) are the most frequently isolated mastitis pathogens from cows with and without a high somatic cell count. This heterogeneous group of bacteria has traditionally been regarded as minor pathogens. Still, the impact and virulence of CNS depends on the species that is involved, as has recently been substantiated by Supré et al. (2010⁴). In the latter study *Staphylococcus chromogenes*, *Staphylococcus xylosus*, *Staphylococcus simulans* and *Staphylococcus cohnii* were the four most frequently isolated CNS-species. Intramammary infections (IMI) caused by *Staphylococcus chromogenes*, *Staphylococcus xylosus* or *Staphylococcus simulans* elevated the somatic cell count (SCC) in a way comparable to *Staphylococcus aureus*, generally accepted as a major pathogen, whereas others like *Staphylococcus cohnii* only caused a very moderate increase, not different from non-infected quarters, in SCC. Since those three moderately pathogenic species might cause some trouble, more research is needed.

One of the final goals of the new research proposal is to outline a more CNS-species-specific prevention and control program. In order to reach that goal, cow- and quarter-level risk factors will be determined for IMI specifically caused by the moderately pathogenic CNS-species present at parturition as well as during the lactation.

A field study will be conducted on ten dairy farms. On each farm, twelve cows and heifers will be randomly selected and included in the study. Two weeks before parturition all teat apices will be swabbed to determine to what extent they are colonized with CNS and whether or not this is associated with IMI shortly after calving. Between 1 and 4 days in milk, quarter milk samples will be collected. As well, data on risk factors at the cow-, quarter- and observation-level for IMI with the moderately pathogenic CNS-species during lactation are available from an existing dataset^a.

A recently conducted study^a indicated that some species mainly cause persistent IMI whereas others are more often involved in transient infections. These findings will be further explored by strain-typing of available isolates by Pulsed Field Gel Electrophoresis (PFGE). Potential sources of infection will be studied at the strain-level (PFGE) as well. Samples of hands and elbows of the milkers, teat apices and teat cups have been collected for that purpose. Finally, isolates from different geographical origins will be compared at strain-level (PFGE). A dominant clonal complex and a representative strain per species for further experimental research will be sought after.

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Interaction between mastitis pathogens during incubation of mastitis samples

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Introduction

Mastitis diagnosis in samples containing a single mastitis pathogen can be done based on their volatile metabolites (Hettinga et al, 2008). With bacterial culturing, however, in approximately 10% of the mastitis cases, two pathogens are simultaneously detected. Because samples are incubated before analysis of volatile metabolites, differences in growth rates and interaction between pathogens may influence pathogen identification. In regular mastitis diagnosis (based on microbial plating), incubation of samples is also frequently used. The objectives of the work performed were: 1) to establish whether a combination of two bacteria inoculated in milk would result in suppression or induction of bacteria, and 2) to determine whether the effect of pathogens on each other could be explained by organic acid production.

Materials and methods

Four pathogens (Gram-positive: *S. aureus* and *S. uberis*, Gram-negative: *K. pneumoniae* and *E. coli*) were provided by the Animal Health Service and grown in BHI broth. Pasteurized milk was inoculated with these pathogens, either alone (10^5 CFU/mL) or in a combination (concentration ratios: $10^5:10^5$ or $10^2:10^5$ CFU/mL). After incubated (24h at 37°C), bacteria were counted using selective media. ¹H-NMR was used to quantify all organic acids in the samples simultaneously. pH of all samples was determined.

Results and discussion

In all experiments, growth of a Gram-positive bacteria was suppressed by Gram-negative bacteria, even in a 1000:1 ratio. Combination of both Gram-negative bacteria resulted in growth suppression of each other. Combination of both Gram-positive bacteria resulted in inhibition of *S. aureus* by *S. uberis*. The highest concentrations of organic acids were observed in samples with Gram-negative bacteria. Both *E. coli* and *K. pneumoniae* produced mainly acetic acid, a potent inhibitor of bacterial growth. *S. uberis* produced lactic acid and *S. aureus* produced only trace amounts of organic acids. The concentration of organic acids produced by these pathogens appears to be related to the inhibition effects seen in combined inocula: the pathogens producing the highest concentrations of organic acids had the strongest inhibitory effect on the other pathogens. The pH of the samples, although correlated to organic acid production, was in itself not enough to explain the differences in growth inhibition.

Conclusions

The results of this study show that mastitis pathogens interact with each other during incubation of milk. In samples containing multiple pathogens, the main pathogen present after incubation depends more on the bacterial specie, than on the bacterial counts in the sample before incubation. The dominant bacterial specie after incubation appears to be related to production of organic acids. These results may also be important for classical microbiological culturing, because identification of pathogens using incubated mastitis samples will also be influenced by differences in growth characteristics and interaction between pathogens in mixed cultures.

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Cow and quarter characteristics associated with teat dimensions

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Introduction

Despite genetic selection towards smaller teats, there is still a considerable variation in teat sizes and shapes between herds and between cows belonging to the same herd (Rasmussen et al., 2003). Still, cows within a herd are traditionally milked with the same milking machine settings. Obviously, these settings are for some of the cows far from optimal. Therefore, some of the adverse effects related to machine milking could be avoided if teat size could be standardized within a herd (Rasmussen et al., 2003). In order to obtain more uniform teat sizes in a herd, knowledge at which level (herd, cow, quarter) most variation resides as well as on the factors potentially related with teat dimensions is needed.

Materials and methods

Teat length and diameters were determined using an objective 2D vision based measuring device developed at ILVO (Zwertvaegher et al., 2010). Data consisted of measurements of 2715 teats from 683 Holstein cows of 15 herds in Flanders from October 2008 to February 2009. All teats were measured prior to milking. Month, parity, lactation stage, milk production near test-day and quarter position were added to the database. Linear mixed regression models were built with teat length and teat diameter at 75%, 50% and 25% of the total teat length, respectively, as dependent variables using MLwiN 2.19. The regression-model building process to identify risk factors involved several steps as previously described (De Vliegher et al., 2004). Herd and cow were included as random effects whereas the different potentially associated factors were included as fixed effects.

Results and discussion

Teat length and diameters at the barrel and the tip significantly vary between quarter positions. Hind teats are shorter and slightly smaller than front teats. Additionally, teat length and diameter increase with parity number and teats lengthen with advancing lactation, corresponding well with previous findings. The observed decrease in teat diameters with lactation stage is not supported by previous studies. Those studies, however, monitored teats over time, whereas we used cross-sectional data. To get more insight in the changes of teat dimensions over time, a longitudinal study is needed. Most of the variation in teat length resides at the quarter level whereas for teat diameters most of the variation resides at the cow level. Consequently, adapted milking installation settings for front versus hind teats and for parity and lactation stage could most probably contribute to better teat condition and milking performances, eventually leading to better udder health. Yet, continuous selective breeding remains essential.

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Effect of oral supplementation of medium chain fatty acids (Aromabiotic®) on udder health, milk production and blood and milk neutrophil viability of dairy heifers and cows in early lactation

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Based on observations from the field, oral supplementation of medium chain fatty acids (MCFA) (Aromabiotic®) seems to improve udder health in dairy cows. Still, results from clinical trials including both treated and control animals are warranted to ascertain the potential efficacy of these lipid molecules. The objective of the present study was to explore the effect of orally supplemented MCFA to heifers and multiparous cows starting 6 to 8 weeks prior to calving on blood and milk neutrophil apoptosis between 1 and 3 days after calving in a double-blinded clinical trial including treated as well as control animals kept under the same management conditions. Twenty-two animals (10 heifers and 12 multiparous cows) were included in the study of which 5 heifers and 6 cows were orally supplemented with MCFA (25gr per day) from 6 to 8 weeks prior to calving on as well as during the first 4 months of lactation. Polymorphonuclear neutrophilic leukocyte viability in both blood and milk was estimated by determining the proportion of apoptotic cells using flow cytometry. Data on composite milk somatic cell count (SCC) and milk yield (kg milk per day) at test-day up till 4 months after calving were available per cow or heifer on a four-weekly basis as part of the Dairy Herd Improvement Program. To evaluate the effect of MCFA supplementation before calving on the blood and milk neutrophil viability shortly after calving, a linear (mixed) regression model with blood or milk neutrophil viability as outcome variables and animal as random effect, when appropriate, was fit. A similar model was fit with the natural log-transformed SCC and milk production (kg/day) as outcome variables. Statistical significance was defined at $P < 0.05$. At the onset of the study, no significant differences in blood nor milk neutrophil viability were found between treated and control animals. In non-supplemented animals, blood neutrophil apoptosis significantly increased between start of supplementation and the first days after calving ($P < 0.001$) whereas no substantial change in blood neutrophil apoptosis could be observed in the MCFA supplemented animals ($P = 0.69$). Similar results were obtained for milk neutrophil apoptosis in multiparous cows. Overall, the proportion of apoptotic milk neutrophil in early lactation was lower in the MCFA supplemented group compared with the non-supplemented group ($P < 0.001$). An identical effect of oral MCFA supplementation on blood and milk neutrophil apoptosis was observed for both heifers and multiparous cows. As was expected based on literature¹, blood as well as milk neutrophil apoptosis shortly after calving was higher in multiparous cows than in heifers ($P < 0.05$). Furthermore, both lactational geometric mean SCC and milk production were slightly higher in the MCFA-supplemented animals (78.000 cells/ml and 39.0 kg milk/day) than in the non-supplemented ones (57.000 cells/ml and 37.3 kg milk/day), although the difference did not reach significance, probably due to a lack of power. In conclusion, oral supplementation of MCFA to heifers and multiparous cows from 6 to 8 weeks before calving suppressed in this study the natural \ddot{o} dipö in the systemic as well as the local innate immunity shortly after calving independently from cowsø parity. The association between MCFA supplementation and neutrophil viability on one hand and the udder health and milk production throughout lactation on the other hand definitely merits further research.

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Better udder preparation, less teat end callosity

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Teat end callosity (TEC) or teat end hyperkeratosis is a localized hyperplasia of the *stratum corneum* of the teat end and is mainly caused by mechanical effects on the teat end. The two main characteristics of teat end callosity are *thickness* (magnitude of the hyperplasia) (TECT) on the one hand, and *roughness* (presence of coarseness) (TECR) on the other hand. As TEC is generally thought to be a risk factor for intramammary infections (Neijenhuis et al., 2001), knowledge about the risk factors for development of TEC is essential for prevention of both TEC and intramammary infections.

The aim of the present study was therefore to screen potential animal- and milk process-related risk factors for TECT and TECR.

In a monthly follow-up during one year on 6 well-managed commercial dairy herds, TEC and potential risk factors for TEC were measured. Teat ends of 10 cows per herd were visualized via digital photographing with a modified digital camera. Visual scoring of both TECT and TECR was conducted on the digital images according to the generally accepted classification system of Neijenhuis et al. (2000). Biological cow-related data (parity, stage of lactation, milk yield at test day, etc.) were derived from the DHI program and milk process data (peak milk flow, duration of milking, duration of blind milking, bimodality of the milk flow curve, etc.) were obtained from the LactoCorder measuring device. The clustered data were analyzed with MLwiN 2.02 to identify risk factors statistically and biologically associated with TEC.

Rear teat ends were likely to have less callus thickness than front teat ends. Over the lactation, TECT was low in the first 60 DIM, peaked between 60 and 120 DIM, and afterwards gradually decreased towards the end of the lactation. Increasing parity and a longer duration of milking were related with a more callus. Also, bimodality of the milk flow curve was associated with increased TECT. Risk factor analysis for TECR is still in progress.

The only risk factor for TECT which could be influenced by the milker is the bimodality of the milk flow curve. The shape of the milk flow curve is a result from interactions between the milker and the physiology of the cow. Optimization of this interaction may have a positive influence on TECT.

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Alert preferences of dairy farmers working with automatic milking systems

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Automatic milking systems (AMS) generate alert lists. Based on these lists, farmers have to decide which cows to inspect for having clinical mastitis. A major complaint is that too many cows are on the alert list while not having clinical mastitis. Research, until now, was mainly aimed at improving detection algorithms (Kamphuis, 2010) or combining different information sources in order to support farmers in their decisions (Steenefeld, 2010). In all these studies, the gold standard for clinical mastitis was defined by researchers. However, there is no consensus on the real gold standard among researchers (Mein and Rasmussen, 2009). And, even when researchers reach consensus, do all farmers agree on that? In this study we will address this problem from the farmer's viewpoint. What type of alerts do they prefer? In order to elucidate the farmer's preferences, about 150 farmers will be interviewed using adaptive conjoint analysis. This method is a marketing tool which is able to rank preferences related to different aspects of a product (in this case alerts). For conjoint analysis a product is thought to have different attributes (product characteristics), with each attribute having multiple levels. Adaptive conjoint analysis starts with determining consumer preferences for each level within each attribute and the importance of each attribute (compared with other attributes). The adaptive conjoint analysis uses this (prior) information and answers on previous questions to formulate a utility for each level of an attribute. After each question the utility value is updated and the next question is formulated using these new utilities. In this way the number of questions is minimized. The questions asked are paired questions; and so preferences for levels of all attributes are compared.

Attributes which will be used are the consequences (for the farmers) of test performance characteristics; sensitivity, specificity and time-window. These characteristics are interdependent and so it is not possible to maximize all of them at the same time. The aim is to find out what farmers perceive as the optimal alert. Asking the farmer questions on what they think about consequences of test characteristics enable us to say something about farmer preferences for the gold standard (i.e. farmer's optimal alert concerning sensitivity, specificity and time-window).

With the results of this analysis, we hope to be able to define a general gold standard or to be able to distinguish two or more groups of farmers with different preferences. These results could be used to develop alert lists that better correspond with the farmer's preferences and for evaluation of CM detection performance.

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Cost and benefits of mastitis management measures

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Costs of mastitis consist of losses (a reduction of output due to mastitis) and expenditures (additional inputs to reduce the level of mastitis). It is expected that for a large proportion of farms there are much avoidable losses. Application of more preventive measures can reduce the losses due to mastitis. In order to make good decisions, it is necessary to provide the dairy farmer with information on the additional expenditures and reduced losses associated with the different decision alternatives. Using estimates of the effects of management measures on the mastitis situation and a cost-calculation tool (Huijps et al., 2008), the net benefit of preventive measures have been estimated (Table 1). Six out of 18 preventive measures showed to have a positive net benefit: blanket use of dry cow therapy, keeping cows standing after milking, back-flushing of the milk cluster after milking a cow with clinical mastitis, application of a treatment protocol, washing of dirty udders and the use of milkersø gloves. Especially for those measures that include a large amount of routine labour or large investments, the reduced losses do not outweigh the additional expenditures.

Table 1. Net benefit (b/cow/year) of implementing mastitis prevention measures on a standard farm .

	Additional expenditures	Reduced losses	Net benefit
Milk cows with clinical mastitis last	37	16	-21
Milk cows with subclinical mastitis last	104	20	-84
Use of separate cloths during udder preparation	26	9	-17
Wash dirty udders during udder preparation	3	9	6
Prestripping	34	9	-25
Use of milkersøgloves during milking	1	9	8
Post milking teat disinfection	31	31	-0
Back-flushing clusters after milking a cow with clinical mastitis	1	11	10
Back-flushing clusters after milking a cow with subclinical mastitis	123	15	-108
Replace teat cup liners in time	13	11	-2
Use of a treatment protocol	7	15	8
Application of blanket dry cow therapy	9	36	27
Keep cows standing after milking	2	12	10
Feed additional dry cow minerals	13	13	0
Prevent overcrowding	23	13	-10
Clean boxes	54	15	-39
Clean yards	51	8	-43
Optimize feed ration	24	13	-11

The advisor cannot expect that measures that are cost effective are always implemented. Reasons for this lack of implementation are: the objectives of the dairy farmer can be other than profit maximisation, resources to improve the mastitis situation compete with other management fields, risk involved with the decision, economic behaviour of the dairy farmer and valuation of the cost factors by the dairy farmer.

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Prevalence and risk factors of udder cleft dermatitis in Dutch dairy herds. Preliminary results.

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Introduction

Udder cleft dermatitis (UCD; ulcerative mammary dermatitis, foul udder) is mostly localized between the forequarters and the abdomen (Beattie and Taylor, 2000; Warnick et al, 2002). Characteristic features of UCD are a moist appearance, necrosis of skin and a foul odor. Udder cleft dermatitis is well known in the field, however relatively little is described in the veterinary literature. Thus far, the etiology and treatment of UCD are unknown or disappointing. Udder cleft dermatitis is seen in all stages of lactation, also in non-lactating cows, but it is more common in older cows. The objectives of this study are to investigate potential risk factors of UCD on cow level and to estimate the prevalence of UCD on both farm and cow level.

Materials and methods

Data were collected from 20 randomly selected dairy farms in The Netherlands. Each lactating and dry cow was photographed from a posterior, lateral and ventral aspect. Photographs were used to determine various measurements and hygiene scores.

Results and discussion

Udder cleft dermatitis (score 3 to 5) was recorded in 5.7% (95% binomial confidence interval: 4.3 ó 7.4) of all animals. Farm level prevalences ranged between 0.0% and 14%. An association existed between both UCD and fore udder attachment and the angle between udder and abdominal wall. Cows with a weak fore udder attachment are more likely to develop UCD. Also, UCD is seen more frequently in cows with a smaller angle between udder and abdominal wall. It is possible that a smaller angle creates an optimal environment for certain microorganisms to colonize, resulting in occurrence of UCD.

References

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