Annual Meeting

DMRW

18 December 2007

Faculty of Veterinary Medicine
Utrecht University
The Dutch Mastitis Research Workers is an independent platform where researchers can present and discuss their work in an informal and confidential environment. This annual meeting was made possible by the 5-yr mastitis program of the Dutch Udder Health Centre and is financially supported by the Dutch Dairy Board.
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Welcome

Dear colleagues,

On behalf of the organizing committee of this first annual meeting of the Dutch Mastitis Research Workers, I would like to welcome you today to Utrecht. We are looking forward to a busy day with as much as 23 presentations on mastitis research going on in the Netherlands and Belgium, the maximum we could handle in one day. In the back of this book of abstracts there even is a number of extra abstracts, we found no room for in the program.

The DMRW meets every three months, during which meetings we have in depth discussions on two or three selected subjects. Today we have a different goal. A whole number of short presentations will be given, with the aim to share the content of ongoing projects, to look for linkages between different projects and to come up with different view points for problems researchers are struggling with.

Although there are several different initiatives and financers for mastitis research in our country, it has been boosted very much by the five year mastitis program. This program is financially supported by the Dutch Dairy Board (PZ), while the initiative was taken by the Dutch Federation of Agriculture (LTO) and the Dutch Dairy Organisation (NZO). This means that most of the work presented here today has been directly paid by farmers. This is unique in the world, and has to be applauded. It therefore is our challenge to pay them back, by translating our findings to the field. Today, however, we are talking among researchers, sharing our knowledge and experiences. Let’s enjoy the possibilities of getting together with such a big group of Dutch mastitis research workers.

I wish you all a very informative and pleasant meeting!

Theo Lam

DMRW
Program

9:00  Coffee and Tea
9:05  Welcome Theo Lam

Epidemiology

9:15 – 9:30  Bart van den Borne
Monitoring and treatment of subclinical mastitis in the Netherlands

9:30 – 9:45  Kasper Hettinga
Detection of Mastitis Pathogens by Analysis of Volatile Bacterial Metabolites

9:45 – 10:00  Gerrit Koop
Factors influencing bulk somatic cell count in dairy goat milk in The Netherlands

10:00 – 10:15  Otlis Sampimon
Evaluation of two phenotypic test kits for identification of Coagulase-Negative Staphylococci isolated from bovine milk samples

10:15 – 10:30  Karlien Supre
Validation of tDNA-Intergenic Spacer PCR for species level identification of bovine Coagulase-Negative Staphylococci

10:30 – 10:45  Richard Olde Riekerink
Risk factors associated with the incidence rate of clinical mastitis in heifers

10:45 - 11:15  Coffee and Tea

Immunology and resistance

11:15 – 11:30  Tine van Werven
Vitamin E supplementation and mastitis reduction in the Netherlands

11:30 – 11:45  Lily Pels Rijcken
Dry cow management in Dutch dairy herds

11:45 – 12:00  Tosca Ploegaert
Parameters for natural resistance in cow’s milk

12:00 – 12:15  Sofie Piepers
Neutrophil viability in dairy heifers

12:15 - 13:15  Lunch

Social science

13:15 – 13:30  Marcel van Asseldonk
Awareness and the value of information in controlling subclinical mastitis

13:30 – 13:45  Chantal Steuten
Effective communication with ‘hard to reach’ farmers
13:45 – 14:00  Jolanda Jansen  
Communication in practice: The role of veterinarians as udder health advisors  

Genetics  
14:00 – 14:15  Jack Windig  
A new udder health index for better breeding against mastitis  
14:15 – 14:30  Yvette de Haas  
The usefulness of breeding to decrease mastitis incidence  
14:30 – 14:45  Jan ten Napel  
Defining new traits from underlying distributions of somatic cell counts  
14:45 – 15:15  Coffee and Tea  

Machine milking  
15:15 - 15:30   Erwin Koenen  
Automatic detection of mastitis using sensors and Internet: A demonstration project  
15:30 – 15:45   Wilma Steeneveld  
Improvement of mastitis detection, pathogen diagnosis and online decision support around treatment for automatic milking systems using additional non-automatic milking system data  
15:45 – 16:00   Claudia Kamphuis  
Inline Somatic Cell Count improves the detection of clinical mastitis in an automatic milking system  
16:00 – 16:15  Erwin Mollenhorst  
Detection of mastitis and abnormal milk with automatic milking systems  
16:15 – 16:30  Francesca Neijenhuis  
Milking and udder health projects  

Economics  
16:30 – 16:45  Kirsten Huijps  
Costs of mastitis and efficiency of management measures  
16:45 – 17:00  Tariq Halasa  
Bioeconomic modeling of Bovine mastitis  

Extra abstracts  
Kees de Koning  
The Dutch Quality System for Milking Machine Maintenance  
Gidi Smolders  
Udder health and farm management on organic dairy farms without antibiotics  
Bert Ipema  
Quarter-controlled milking
Monitoring and treatment of subclinical mastitis in the Netherlands

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²-GD Animal Health Service Ltd, Deventer
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Introduction
In 2005, the Dutch Udder Health Centre (UGCN) was founded to execute a national five-year udder health program to decrease mastitis in the Netherlands. To monitor occurrence of subclinical mastitis (SCM) during this program, a baseline survey was conducted to assess the prevalence of SCM preceding this program and to set a definition of SCM incidence rate. Subsequently, two interventions were started: 1) lactation treatment at the cow level and 2) udder health emphasis at the level of the private practice.

Materials and methods
Cow SCC of all cows from all Dutch dairy herds participating in the test day recording were used in the baseline survey to estimate prevalence and incidence rate of SCM. Different thresholds and patterns from below to above a threshold were evaluated. To evaluate the first year of the udder health intervention program, bulk milk SCC were collected from herds belonging to 10 private practices that started with the five-year program. Herds belonging to 20 private practices, not participating in the program, were used as controls. In the clinical trial, cows with a first or second elevated cow SCC and a positive bacteriological culture are randomly allocated to lactation treatment to observe bacteriological cure of acute SCM.

Results
The average SCM prevalence was 23.3 in 2003 and 2004, using the currently used threshold in the Netherlands. SCM incidence rates depended heavily on the chosen threshold and on the used pattern from below to above the threshold. Bulk milk SCC of farms belonging to 10 private practices participating in the 5-year udder health program showed a significant lower bulk milk SCC for herds participating in study groups compared to herds not participating in those study groups (figure 1). The clinical trial will give results on bacteriological cure in treated cows with acute SCM. Indirect benefits such as reduced culling, reduced occurrence of clinical mastitis and reduced milk losses will be evaluated for both treated and untreated cows to study economic feasibility of lactation treatment of acute SCM.

Discussion
The currently used threshold of SCM in the Netherlands was chosen to monitor SCM prevalence during the five-year udder health program. The pattern of change of two consecutive cow SCC below threshold to one cow SCC above threshold was chosen to identify a new SCM infection in the udder health program. The results regarding bulk milk SCC in the first year of the five-year udder health program are promising for the rest of the project. However, farms not participating in the study groups (intervention-passive) did not show a decrease in log BMSCC. The challenge for the next coming years will therefore be to trigger these farms to improve udder health at their farms. Treatment of subclinical mastitis may depend on several pathogen factors. Antibiotic resistance testing and strain typing will therefore be used to differentiate cure rates for different antibiotic resistance levels and for different strains of the same pathogen.

Figure 1. Bulk milk SCC in herds participating in study groups (intervention-active), herds not participating in study-groups (interaction-passive) and herds in control practices (control) in the year preceding the program (2004) and during the beginning and the remaining part of the program (2005-2006).
Detection of Mastitis Pathogens by Analysis of Volatile Bacterial Metabolites

Kasper Hettinga

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Introduction
Mastitis with or without clinical symptoms is most often caused by bacteria. Determination of the mastitis causing pathogen is of great interest, both for choice of treatment as well as for possible measures that have to be taken on the farm to prevent the spread of mastitis. Currently, determination of the pathogen is done with classical microbiological methods. The main disadvantage of these microbiological methods is that they are time-consuming. Faster and more accurate methods of pathogen detection are advantageous, because farmers are earlier able to choose an optimal treatment.

In microbiology, screening of volatile bacterial metabolites for detection and/or classification purposes is well known. The detection is based on the fact that all microorganisms have their own group of enzymes, producing their own range of volatile metabolites (Turner and Magan, 2004). In our study, clinical mastitis samples were examined with classical microbiological methods and by headspace analysis for their volatile metabolites, comparing the results of both methods.

Materials and methods
Fifty milk samples from cows with clinical mastitis were selected from the bacteriological diagnostic lab of the Dutch Animal Health Service. Samples had been screened for the presence of bacteria. If one of 5 pathogens of interest (Staph. aureus, Coagulase-negative staphylococci (CNS), Strep. uberis, Strep. dysgalactiae, or E. coli) was cultured, the remainder of the sample was stored at -20°C before further use. 10 milk samples from cows with SCC<75.000 and no clinical mastitis signs were used as control.

Analysis of volatile metabolites occurred only after bacteriological screening; all milk samples had thus been incubated overnight at 37°C. Milk samples of 5 mL were heated to 60°C in a closed vial before extraction of the volatile metabolites using Solid Phase Microextraction (SPME). Next gas chromatography/mass spectrometry (GC/MS) was used to separate, identify, and quantify the volatiles.

Results
A maximum of 19 different volatile metabolites were identified and quantified. Control samples could easily be differentiated from mastitis samples. Of the pathogens, Staph. aureus produced the widest range of unique volatile metabolites and could thus be easily identified. The patterns of metabolites of CNS and E. coli could also be used to identify them. The two streptococci did not differ between each other, but were, as a group, different from the 3 other pathogens.

A neural network was trained to classify the different samples according to the pathogen. The results of this network can be found in this table (results based on cross-validation).

<table>
<thead>
<tr>
<th></th>
<th>Percent correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 pathogens separately</td>
<td>66%</td>
</tr>
<tr>
<td>4 pathogens (both streptococci as one group)</td>
<td>93%</td>
</tr>
<tr>
<td>Gram-negative vs. Gram-positive vs. Control</td>
<td>96%</td>
</tr>
</tbody>
</table>

Conclusions
Volatile metabolites found in milk from uninfected quarters differed significantly from metabolites found in milk from infected quarters. Additionally, different pathogens were found to differ in the formation of volatile metabolites. These differences could be used by a neural network to identify the mastitis pathogen. These results show that mastitis pathogen classification using volatile bacterial metabolites looks very promising.
Factors influencing bulk somatic cell count in dairy goat milk in The Netherlands

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Introduction
Little is known about the meaning of somatic cell count (SCC) in goat milk. SCC in goat milk seems to be generally higher than in cow milk and is influenced by more factors than only inflammation of the udder. Since in recent years the Dutch dairy goat sector has grown rapidly, there is an increasing demand for knowledge on dairy goat production. This study aims to describe the factors that influence the BSCC in goat milk.

Materials and Methods
Bulk milk somatic cell counts (BSCC) of Dutch dairy goat farms are recorded since 2001. Questionnaires are to be send to all goat farmers in The Netherlands. Main topics of the questionnaire are milking technique, mastitis and mastitis prevention, animal health and feeding management. The correlations between farm characteristics and the BSCC are calculated.

Results
The questionnaires are to be sent to the farmers in January 2008, so no results are available yet. Some of the BSCC data though are already collected. A first impression of these data is given in the figure. The mean BSCC for all farms shows a clear yearly pattern. BSCC seems to rise before or during the kidding season. There is relatively much variation between the farms.

Discussion
It is known that an increase in SCC in late lactation is at least partially physiologically [1]. This may explain the increase in BSCC during the winter month (December, January). The large differences between the farms though give rise to the idea that other factors may influence SCC as well. Analysis of the questionnaire results and other BSCC data are to reveal some of them.

References
Evaluation of two phenotypic test kits for identification of coagulase-negative staphylococci isolated from bovine milk samples

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Introduction
Coagulase-negative staphylococci (CNS) are a heterogeneous group of organisms. The genus \textit{Staphylococcus} consists of more than 40 species. To assess the pathogenic significance of individual CNS species and to develop species-specific management practices, accurate species identification is needed in epidemiological investigations. Commercial test kits for identification of CNS based on phenotypic tests, such as Api Staph ID 32 and the Staph-Zym test, are relatively easy to use. The aim of this study was to measure the accuracy of two frequently used phenotypic methods, the Api Staph ID 32 and the Staph-Zym test, for identification of CNS, isolated from bovine milk samples in comparison with sequencing of housekeeping genes.

Results
A total of 180 CNS isolates were analyzed. The rpoB-gene sequencing identified 173 (96.1%) of the 180 isolates, 5 samples contained too little DNA. One isolate was identified as \textit{Streptococcus uberis} and one isolate was identified as \textit{Moraxella osloensis}. Api Staph ID 32 (cut-off \geq 90\% probability) identified 92 of the 173 (53.2\%) isolates, with 71 of 92 (77.2\%) identified correctly. In 43 of 173 isolates (24.9\%) additional tests were needed. Staph-Zym test was able to identify 157 of the 173 (90.8\%) isolates, with 53 of 157 (33.8\%) identified correctly. In 113 of 173 isolates (65.3\%) additional tests were needed.

Table 1. Identification of CNS species with the Api Staph ID 32 and the Staph-Zym test, compared with sequencing of the rpoB gene.

<table>
<thead>
<tr>
<th>CNS species</th>
<th>Sequencing rpoB gen</th>
<th>Api Staph ID 32\textsuperscript{1}</th>
<th>Staph-Zym\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CI</td>
<td>% CI</td>
</tr>
<tr>
<td>\textit{S. chromogenes}</td>
<td>63</td>
<td>23</td>
<td>36.5</td>
</tr>
<tr>
<td>\textit{S. cohnii subsp. cohnii}</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>\textit{S. epidermidis}</td>
<td>23</td>
<td>23</td>
<td>100.0</td>
</tr>
<tr>
<td>\textit{S. equorum}</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>\textit{S. fleuretti}</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>\textit{S. haemolyticus}</td>
<td>9</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>\textit{S. hyicus}</td>
<td>9</td>
<td>8</td>
<td>88.0</td>
</tr>
<tr>
<td>\textit{S. simulans}</td>
<td>9</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>\textit{S. warneri}</td>
<td>13</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>\textit{S. xylosus}</td>
<td>15</td>
<td>13</td>
<td>86.7</td>
</tr>
<tr>
<td>Other CNS species\textsuperscript{*}</td>
<td>12</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>71</td>
<td>41.0</td>
</tr>
</tbody>
</table>

\textsuperscript{1}CI=correct identified, MI=misidentified, UI=unidentified.

Conclusion
Both tests were relatively easy to perform, but both had a low level of agreement with rpoB gene sequencing in identifying bovine CNS species. The Staph-Zym test needs much more additional tests which adds extra costs and labor.
Validation of tDNA-intergenic spacer PCR for species level identification of bovine coagulase-negative staphylococci

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Introduction
In many European dairy farms that have adopted the 5- and 10-point mastitis prevention programs, the relative importance of coagulase-negative staphylococci (CNS) has increased. The increase in CNS prevalence relative to traditional major pathogens, combined with changes in limits for bulk milk somatic cell count penalties and the fact that CNS are causing clinical mastitis also, warrant reconsideration of their historical designation "minor pathogen". On the other hand, protective characteristics of CNS have been reported. The confusion can partly be explained by the lack of (accurate) species identification, which is a prerequisite for elucidating the importance of CNS. Current identification methods are largely phenotypic and based on human reference strains, but this may not be suitable for bovine CNS. Within the scope of our field study concerning CNS on dairy farms, we have been searching a molecular method that is cheap, rapid and easy to perform. At our laboratory, tDNA-intergenic spacer PCR (tDNA-PCR) is available but has never been validated for identification of bovine CNS. In this study, we have updated tDNA-PCR for identification of bovine CNS species by extending the current library of the technique, followed by comparing the results with sequencing of the rpoB-housekeeping gene.

Materials and methods
Isolates: For the extension of the tDNA-PCR library, 146 CNS isolates originating from bovine milk (94) and teat apices (52) were used (results not shown). To validate tDNA-PCR, 148 bovine CNS (100 and 48 from bovine milk and teat apices, respectively) were analysed and tDNA-PCR results were compared with gene sequencing. Techniques: tDNA-PCR1,2,4 and sequencing of the rpoB-gene3 were performed as described with small modifications. When there was no amplification with the rpoB-primers or less than 97% homology with reference strains was seen, additional cpn60-sequencing and if not sufficient 16S-sequencing, was performed.

Results and discussion
When studying the impact of different CNS species on performances in dairy cattle, an accurate identification technique is required. Although no single test can offer fully reliable identification of bacterial species, gene sequencing is often seen as the gold standard. Unfortunately, the high cost and its labour intensiveness limit its use in large field studies for most routine laboratories. Phenotypic methods on the other hand are usually cheaper but lack accuracy. tDNA-intergenic spacer PCR is a rapid, low-cost and easy to perform technique that has a high reproducibility if capillary electrophoresis is available2. The results of this study show that tDNA-PCR could be a good alternative for gene sequencing. After updating the current library, the overall agreement (isolates from milk and teat apices) between tDNA-PCR and gene sequencing was 97.5%. When focussing on milk samples and teat apices separately, 96.6 and 100% of the identifications agreed in both tests, respectively. Overall, a high number of CNS-isolates could not be identified with the gold standard (gene sequencing: rpoB, cpn60, 16S), especially isolates originating from teat apices (20.3%). Possible explanations could be the presence of undefined species on teat apices, or strain differences between isolates from different origins. Still, availability of a complete reference database is a prerequisite and could be the bottleneck. Additional sequencing of the tuf-gene might give a definite answer (in progress). To conclude, tDNA-PCR will be a useful tool for our field study aiming at elucidating the relevance and epidemiology of CNS in dairy cattle, and its use in other laboratories should be promoted.

Acknowledgements
This research is funded by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen, grant n° 61459). The authors acknowledge the Dutch Udder Health Centre, Deventer, The Netherlands, for financially supporting part of the analyses.

Risk factors associated with the incidence rate of clinical mastitis in heifers

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18 december 2007

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Introduction
Mastitis prevention programs focus in general on the whole herd and not specifically on heifers. Dairy managers consider heifers being free of intramammary infections (IMI). Heifers, however, are the future of the herd. Incidence rate of clinical mastitis (IRCM) of heifers is higher than that of older cows shortly after calving in studies in Canada and the Netherlands. Heifers have an IMI prevalence of up to 50% in the third trimester of gestation and high IMI prevalence of coagulase-negative staphylococci and *Staphylococcus aureus* shortly after calving. An elevated somatic cell count (SCC), which is associated with IMI, in early lactation heifers will negatively impact production during the first lactation, and stresses the importance of mastitis prevention in heifers in this period. The aim of the presented study is to identify pathogen-specific risk factors associated with IRCM in heifers at animal and herd level.

Materials and methods
In total, 500 herds will be selected from herds which previously participated in study and discussion groups organized by their veterinarians and supported by the Dutch Udder Health Center. Each herd will receive a questionnaire containing questions related to heifer mastitis prevention management. Approximately 300 herds are expected to return a completed questionnaire. Based on this, participants will be selected, while validity of the results of these questionnaires will be monitored. Participating farmers will be asked to record cases of clinical mastitis of both heifers and cows, during a one year period. A second questionnaire will be conducted after the study period. To assess mastitis patterns in the herds, clinical mastitis and SCC data over the last few years will be analyzed, as well as the predominant type of pathogen in each of the herds. For the latter aim, up to twelve milk samples of heifers with clinical mastitis per herd will be collected by the farmers. Based on culture results it can be decided whether pathogens were mainly environmental organisms (such as *E. coli*, or predominantly contagious (such as *S. aureus* or if there were approximately equal proportions of both, as has been described by Green et al. (2007). Association of risk factors with IRCM will be estimated using correspondence and Poisson regression analyses. This project will commence early 2008.

Reference
Vitamin E supplementation and mastitis reduction in the Netherlands

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Introduction
Vitamin E is a fat soluble vitamin with a strong antioxidant function. It has become clear that the supplementation of 1000-3000 IE vitamin E per day has a positive effect on udder health (Smith et al., 1997). A meta analysis of fourteen papers showed a decrease in the number of cases of clinical mastitis as well as a drop in the somatic cell count in cows that were supplemented with vitamin E (Moyo et al., 2004).

A lot of research has been done about vitamins and minerals since they were discovered. However it is still hard to find numbers on the exact needs for vitamins and minerals of the modern dairy cow in the Netherlands. Most of the research of the last decades has been done in the United States and Canada. Management and feeding strategies of these countries are hard to compare to the dairy industry in the Netherlands. In a pilot study 100 blood samples from 46 different farms in the Netherlands showed that 31% of the cows that were orally supplemented with vitamin E during the dry period and 63% of the non-supplemented group had vitamin E blood levels below 7.4 µmol/l (reference value).

Aim of the study is to measure the effect of orally vitamin E supplementation during the dry period on (sub)clinical mastitis under Dutch field conditions.

Materials and methods

Farms
Five Dutch farms were selected for the field study based on the following inclusion criteria:

- At least 100 cows with a production between 8000 – 10,000 kg /cow/year
- % cows with clinical mastitis at least 30 %
- No robot
- Ability to house the dry cows in two different groups, or four different groups in case of two dry cow groups (far-off, close-up)
- Dry cow period of at least 6 weeks

Study design (double blind)
From each farm between 50 – 60 cows will enrol into the study. Cows are randomly selected to belong to group A or B. One group will be fed 100 mg/cow/day of a regular dry cow mineral mix containing 150 IE vitamin E and the group will be fed 100 mg/cow/day of the same mineral mix, but containing 3000 IE vitamin E. The cows will be housed inside during the study.

Blood sampling
Blood samples of each cow will be taken on -8, -4, -2 weeks prior to calving, within 24 hours after calving and on the same day as her first milk test day (MPR). Additional blood and milk samples will be taken from cows with mastitis within 24 hours after onset of the mastitis. Samples are taken to the laboratory to be stored at –80°C until analysis follows at the end of the field study.

Blood analysis
Blood samples will be analysed for vitamin E, cholesterol, GSH-Px, BHBA, total lipids, and NEFA.

References
Dry cow management in Dutch dairy herds

Lily Pels Rijcken

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Introduction
The dry period is a well known risk period for new intra mammary infections (IMI), as well as a period of continuation of chronic IMI (Bradley & Green, 2004, Robert et al, 2006). The management of transition cows (from lactating to dry to lactating again) aims to optimize the cows’ health and production capacity, and is very focused on feed regimes and IMI control (Dingwell et al, 2003).

Recent Dutch research showed that 23% of the cows started the dry cow period with a SCC > 250.000 cell/ml and 40% of these animals started the new lactation with again a SCC > 250.000 cell/ml. On the other hand almost 16 % of the cows entered the dry cow period with a low SCC but started the lactation with a new infection (GD Monitor Rund 2007). In addition, the overall management of transition cows influences the risk of clinical mastitis post partum (pp), as well as the risk of many other health disorders pp.

Many Dutch farmers use dry cow therapy, either blanket or selective, and most Dutch farmers are very aware of the importance of transition cow management. However, the importance of each separate management practice for the reduction of (sub)clinical mastitis pp is not easily quantified, because observational studies generally report on many risk factors at the same time. In addition, not all reported risk factors can be influenced by the farmer (i.e. parity, herd size) and not all factors are relevant for the Dutch situation (i.e. full pasture systems).

This study aims to provide practical advices on dry cow management for the Dutch situation. An update and quantification of dry cow management factors will be made, including dry cow treatment.

Materials and methods
Data collection is taking place on the management of approximately 300 individual transition cows within five farms. The farms are visited weekly and data collection is integrated in a field study on the effect of Vitamin E on mastitis. The collection of data will be finalized around the end of March 2008.

Cow level factors cover test day measures over the last lactation (milk, fat, protein, SCC, lactose, BSK & LW), clinical mastitis status over previous lactations, kg milk per milking during the three weeks before drying off and three weeks after calving, dry cow treatment at dry off, BCS and teat quality during transition, length of the dry period.

Management factors per cow cover feed management and housing during the different phases of transition, method of enforced milk production reduction prior to dry off, cleanliness scores of surrounding cows and housing, mastitis occurrence within the dry cow group, level of crowding in the dry cow group. Additionally, management factors around parturition are scored, including the udder hygiene, management of the firstcolostrum milkings, calf suckling, milk leakage and udder edema.

The analysis of these data will include descriptive analysis on detailed dry cow management in 300 Dutch cows from five farms. Finally, multivariable statistical analyses will be carried out for cohorts with and without certain risk factors, and for identified cases (mastitis pp) and controls (no mastitis pp).

References
Parameters for natural resistance in cow’s milk

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Mastitis is an important problem in dairy farming. Among many other factors, natural resistance of the cow can play a role in preventing and fighting mastitis. The hypothesis of this project is that immune parameters of natural resistance of cows can be measured in milk and that these parameters are correlated to the capacity of animals to offer resistance to infections that lead to health disorders, of which mastitis is one. The project started with a literature review on the potential immune parameters and the possibility to measure them in milk. Based on this a set of promising parameters is selected and tests are developed to measure these parameters. The humoral (soluble) parameters that will be measured include cytokines (messenger molecules of the immune system), like TNFα (inflammatory), IFNγ (regulatory) and IL10 (anti-inflammatory), and antibodies against different pathogen associated molecular patterns (PAMPs), like LPS and LTA from different bacteria. The different immune cells in blood and milk will be characterised (FACS analysis of CD markers; labelled antibodies against specific cell receptors) as well as functionally tested (secretion of cytokines and production of Reactive Oxygen Species (ROS)). The characterisation will be performed to study if differences in cell populations are related to the incidence of mastitis. Reactive Oxygen Species are important in the killing of phagocytised bacteria and a test has been set up to measure this ROS production after in vitro stimulation of cells with PAMPS. An animal experiment will be done very soon to study repeatability within and variation between cows of the different parameters and the relation between milk and blood. Samples will be taken from in principle healthy cows (no clinical signs), so it will be more an observational study and not an infection trial. This is the approach of the whole project.

The project is linked to the Milk Genomics project, where from 2000 cows three milk samples were taken. One milk sample of each cow will be used to measure humoral immune parameters. Much genetic, health and production information is present and can be linked to the immune parameters that will be measured. In this way the effect of genetics on natural resistance can be investigated. Eventually, a field experiment on different farms will be performed to verify the relation between resistance and health and to study the effect of environmental circumstances.

The TNFα, IFNγ, and (natural) antibody detection tests and the cell differentiation and ROS-test have been setup. TNFα and IFNγ were tested on several milk, plasma and cell culture supernatant samples and showed to be a sensitive test, and showed differences between cows. The ROS-test shows to be a good test for isolated cells. Some data of this test will be presented.
Neutrophil viability in dairy heifers

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Earlier and recent evidence showed that neutrophils are of major importance in the non-specific first line immune defence of the mammary gland. Variations in the viability/functionality of blood and milk neutrophils may therefore at least partly explain differences among dairy cows or even among quarters in the resolution of intramammary infections (IMI) and in the resistance to new ones. Bovine blood neutrophils can be easily identified flow cytometrically because of their size and granularity compared to other blood cells. To explore the role of neutrophils in the immunity of the mammary gland under field conditions, a validated method enabling the identification of milk neutrophils and the quantification of their viability in both low (healthy) and high (infected) somatic cell count (SCC) milk is still lacking. Whereas differential leukocyte count methods are available, none of these allows a simultaneous and unequivocal analysis of the milk neutrophil viability.

In our current research, a flow cytometrical method for the identification of milk neutrophils and the simultaneous quantification of their viability under field conditions was optimized and validated. Differentiation of milk neutrophils from other milk cells was performed by indirect fluorescent labelling using a primary anti-bovine granulocyte monoclonal antibody (Ab) and an Alexa 647-labelled secondary Ab. Neutrophil counts were obtained flow cytometrically based on their cytoplasmatic granularity and CH138A-positivity, following confirmation of the adequate sensitivity and specificity of this staining by fluorescence microscopy. Additional labelling with annexin-V-FITC and propidium iodide was used to determine the milk neutrophil viability. Thirty quarter milk samples were run in parallel and six repeated measurements were performed to assess the three-color methods’ repeatability and the instrument stability.

The method greatly facilitated the unequivocal distinction of labelled neutrophils from other milk cells and from autofluorescent particles present in milk. Flow cytometrical quantification of the neutrophil counts, and the viable, apoptotic and necrotic subpopulations gave repeatable results with agreement indices varying between 0.64 and 0.84. The average coefficient of variation for repeated measurements in identical samples ranged between 4.0 and 9.7%. Fluorescence microscopical verification of the CH138A staining pattern showed both a high sensitivity (90.9%) and specificity (92.3%).

As flow cytometrical analysis allows rapid processing of a large number of samples, the technique is useful for future epidemiological studies focussing on the innate immunity of the mammary gland. Currently, periparturient blood and milk neutrophil viability of 82 dairy heifers and 328 quarters are evaluated. Considerable variations in blood and milk neutrophil viability around calving have already been detected. The variation mainly resides at the heifer and quarter level, indicating that future research should focus on heifer and quarter level rather than on herd level. Several heifer level variables such as supplementation of minerals/vitamins, calving season and age at calving are associated with neutrophil viability around parturition. To what extent a higher neutrophil viability at the heifer- and/or quarter level is associated with a heightened resistance against IMI or with a more effective bacterial clearance definitely warrants further research.
Awareness and the value of information in controlling subclinical mastitis

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Adoption and implementation of more efficient subclinical mastitis control practices at farm level is an action of behavioral change, which is notoriously difficult to achieve and sustain, even when studies report substantial production and economic losses. Providing farmers additional information on a regular basis may motivate them to implement more efficient and effective control practices. The objective of this study is therefore to identify whether farmers are aware of production losses and to determine whether incorporating loss estimations into the monthly milk production records is valuable to them.

The conceptual behavioral model (Figure 1) explains the implemented subclinical mastitis control practices. The pathway differentiates between intention and action and describes the influence of perception, risk attitude and farm specific factors on obtaining intent. Besides intent, also efficient control strategies need to be feasible before behavior change can be expected. The value of information notion stipulates that information adds to the knowledge of the person receiving it, enabling him to make improved decisions. Within this framework, additional information will make the decision maker more aware of an event. A farmer who knows that subclinical mastitis causes production inefficiencies can be motivated to act. In the current research an information tool that provides objective loss estimations is incorporated into the monthly milk production records and it is tested whether this alters his intent to reduce cell count.

Figure 1: A conceptual behavioral model to mimic the road map to subclinical mastitis control.

A total of 20 dairy farmers enrolling in the monthly milk production recording will be interviewed. At the farm visit, the farm-specific and cow-specific production and economic losses are appended to the monthly milk production recording of the previous month. Initially, losses and strategies at the whole-farm level are discussed before exploring the control measure applied for each individual attention cow with increased somatic cell count. Thus also the perceived deprived production of attention cows, the associated loss and the chosen current control practices are listed. By eliciting whether intentions and actions to implement control strategies alter, the impact of the provided information is ascertained.
Effective communication with ‘hard to reach’ farmers.

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Motivation
Two years after the start of the Five Year Mastitis Control Program of the Dutch Udder Health Center (UGCN), indications are that a substantial part of the farmers is still hard to be reached by the current information on udder health they receive from their veterinarian and the UGCN. The aim of this study is to provide insight in this group of farmers. What are their characteristics? What is their view on the mastitis issue and the information that reaches them? What are their needs? How do they describe their relationship with their veterinarian? What does motivate them to change their behaviour. Gaining more insight in these questions will help the UGCN in the development of more effective communication instruments and strategies to reach this group of farmers.

Method
In the period Oct. 2007 - Nov. 2007, ten interviews were done with ten veterinarians, all collaborating with the Five Year Mastitis Control Program of the UGCN. Veterinarians were asked about their (changing) role in advice on udder health, about the way they communicate with farmers who do not seem to be very interested in their advice and/or not very motivated to apply this in their daily practice. The veterinarians were asked to describe this group of farmers. From every veterinary practice, three dairy farmers were selected who the veterinarian considered to be difficult to approach with advice on udder health improvement. These thirty selected farmers are interviewed in the period Nov. 2007 - Feb. 2008. The three main topics of these extended semi-structured interviews are 1) description of the farm and the farmer, 2) attitude and behaviour: to what extent does the farmer perceive mastitis as a problem, what does he do in his daily practice to prevent mastitis, 3) information sources and social environment: who or what are his main information sources, does the farmer feel that the advice he gets is useful and fits in his daily practice, what is his need for information and how is the interaction with his veterinarian.

Preliminary results
At the moment of writing, the interviews with the veterinarians were finished. The results show that ‘hard to reach’ farmers are not equally perceived by the veterinarians. Based on their specific perception of ‘hard to reach’ farmers, they describe groups of farmers within this category. Some are, for instance, easy to approach, but just refuse to do anything with the information they are offered. Preliminary results of the first interviews with farmers will be presented during the meeting.
Communication in practice: The role of veterinarians as udder health advisors

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Introduction
Preventive as well as treatment programs of mastitis based on the implementation of management practices sometimes fail for reasons that are not immediately understood. A study of dairy farmers of the Dutch Udder Health Centre (UGCN) showed that farmers perceive the veterinarian as the most important and most frequently contacted person regarding udder health. As such, the veterinarian could play a significant role in a mastitis control program. Results of the mastitis control programs in the Netherlands and other countries show that study groups led by veterinarians are an effective way to improve udder health on dairy farms. However, the participating veterinary practices differ substantially in the percentage of farmers participating in study groups. This may indicate that the role of veterinarians as udder health advisors differs between veterinary practices, but also between veterinarians. These differences may be due to differences in perception of the role as advisor by veterinarians. Questions arise such as: What are opportunities and what are limitations for veterinarians as effective udder health advisors? Understanding of the role of the veterinarian will provide a lead for further improvement of udder health programs.

Material and Methods
Both, quantitative and qualitative data of participating veterinary practices are used to understand the role of the veterinarian. First, a quantitative baseline survey among 93 veterinarians gave insight in the perceptions of veterinarians on national level. Second, group interviews with veterinarians in 10 practices were held to explore opportunities and limitations on veterinary practice level. Third, semi-structured interviews with 10 veterinarians from 10 other participating practices gave insights in the perception of the individual veterinarian.

Results
The results showed that veterinarians perceive the improvement of udder health as their responsibility. They responded to be highly motivated to work on prevention of mastitis and they positioned themselves as suitable to communicate with farmers about udder health. However, when it comes to execution of this role as advisor, veterinarians indicate they find it hard to behave as such, and to ‘sell their knowledge’. Currently, their advice seems to be mostly demand-driven; when a farmer takes the initiative, it is easier to give advice. Results show that veterinarians in general feel insecure about their communication skills and their capacity as a professional advisor towards farmers who do not perceive a problem. In addition, results suggest that it is difficult for veterinarians to come to a mutual agreement about udder health within their practice.

Conclusion
Although “on paper” veterinarians seem to be the best udder health advisors, in daily practice differences exist regarding motivation and communication skills of veterinarians, and management of veterinary practices. Taken these differences into account, veterinarians could transform their limitations into opportunities by investing in a customer oriented pro-active approach as an individual veterinarian, but also as a practice.
A new udder health index for better breeding against mastitis

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Breeding is an important tool to decrease mastitis incidence. Efficient breeding will result in a permanent change in the cows ability to resist and recover from mastitis infections, despite a high milk production. Breeding values play an important role in the selection of bulls for future generations, both for breeding companies and for farmers choosing the sires of their cows. Breeding values of several combinations of traits are evaluated in an index. Here we evaluate the efficiency of indices for the combination of clinical mastitis (CM) and subclinical mastitis (SCM) based on somatic cell counts (SCC). We also evaluate the gain in efficiency if direct information on CM is used.

Information for breeding against mastitis can come directly from registration of mastitis, and indirectly from related traits such as SCC. Current breeding is based on lactation average SCC alone. Alternative SCC-traits have been defined. We looked at 15 such traits in three sets: SCC over a restricted lactation period (early vs late), SCC-traits evaluating excessive SCC (e.g. number of SCC test days above threshold) and patterns of peaks in SCC. For different combinations of SCC-traits the accuracy of an index derived for these traits was determined. The accuracy indicates how well the index predicts the breeding value for the breeding goal, which in our case was defined as reduction of both SCM and CM in equal amounts.

If only information on SCM was used to compose the index, its accuracy was 64% (Table 1). Combining SCM with SCC early and SCC late in lactation considerably improved the accuracy to 85%. Further improvement came with the addition of Suspect (i.e. whether an SCC above 150.000 occurred in the lactation) and Seriousness (i.e. the fraction of test days in a lactation above 150.000). The addition of the presence of peaks added another 1% to the accuracy of the index. All other SCC-traits had hardly any influence on the accuracy. If all traits except CM were used to compose the index the accuracy was over 91%, whereas the currently used lactation average SCC has an accuracy of 72%.

Table 1. Accuracy of estimated breeding values (EBV) predicted by udder health indices composed of different traits. The accuracy is for a bull with 120 offspring with data on all traits, except for clinical mastitis (CM) which varied in number of offspring with information.

<table>
<thead>
<tr>
<th>No.</th>
<th>Data used</th>
<th>No. of offspring with data on CM</th>
<th>Gain relative to no CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>Only (S)CM</td>
<td>0.64</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>1 + Late and Early SCC</td>
<td>0.85</td>
<td>0.89</td>
</tr>
<tr>
<td>3</td>
<td>2 + Suspect, Seriousness</td>
<td>0.90</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>3 + Peaks</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Full set</td>
<td>0.91</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Addition of CM-information further improved the index. However, this improvement was relatively low for indices with 4 or more SCC-traits. The accuracy of an index composed of SCM, early SCC, late SCC, Suspect, Seriousness and Peaks improved by 1.5% if all offspring had information on CM. Thus setting up an information system for CM of all cows in the Netherlands will improve breeding for mastitis resistance only slightly, but using an index composed of 5 SCC-traits will improve breeding against mastitis considerably.
The usefulness of breeding to decrease mastitis incidence

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Introduction
Clinical mastitis (CM) is affected by a number of factors such as pathogenicity of micro-organisms and management factors, like treatment and prevention strategies. But also by cow factors like conformation and immunological performance of the cow, or in other words, the genetic predisposition of the cow. This makes genetic selection another strategy to reduce the incidence of CM. The advantage of reducing CM by selection is that it results in a permanent change in the genetic composition of the dairy herd. Genetic selection can, however, only be used if genetic variance is present in the dairy population. The aim of this study was to determine (1) presence of genetic variation among bulls and among cows, and (2) trends in breeding values (BVs) over the years.

Results
Despite the low heritability for CM and the absence of direct CM-information, genetic variation among bulls was determined. For parity 1, the BVs for CM of bulls range from -5% to +5% around the population mean. For parities 2 and 3, the BVs range from -9% to +6% around the population mean. This indicates that an efficient selection against mastitis is possible. Slightly less genetic variation is shown for the BVs for CM of cows. Their estimated BVs are closer to the population mean, due to the lower reliability of their BVs, because of less offspring per cow.

The estimated average BVs of CM for cows show small differences between parities (Figure 1). This indicates that it seems that with the current breeding strategy, the incidence of CM in parity 1 decreases slightly since 1994, and the incidences of CM in parities 2 and 3 slightly increases since 1995. The estimated BVs of CM for bulls show many fluctuations between the years (Figure 1). The BVs for CM in parity 1 seemed to have increased between 1993 and 1994, and decreased slowly since then. For parity 2 and 3 no trend seems to be present.

![Figure 1](image-url)

Figure 1. Trend in average breeding value (BV) for clinical mastitis (CM) in parity 1 to 3 of Holstein-Friesian sires and cows. The average estimated BV for sires born in a certain year, with at least 100 offspring in milk, is shown. The average estimated BV for cows with records in the national milk recording, born in a certain year, is shown.

Conclusion
The absence of a real clear trend indicates that the current breeding strategy did counteract the negative effect of selection on higher milk production on the incidence of CM. However, a decreasing trend was not seen either, indicating that there is room for improvement for example by a more efficient mastitis index.
Defining new traits from underlying distributions of somatic cell counts

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From biology we know that individual quarters are in one of a number of distinctive health states, such as ‘uninfected’, ‘infected’ and ‘recovering from infection’. Somatic cell count (SCC) of an individual quarter is strongly dependent on the health state of the quarter. The objective of this study was to evaluate whether these states can be found and utilized in the distribution of SCC across quarters (cow-SCC), as commonly available in practice.

A dataset with 316,426 records on 41,567 cows on 407 farms was analyzed. The statistical mode of the distribution (the peak) was the same, but the means varied. This was confirmed in a wide range of subsets of data; i.e. by parity, stage of lactation or previous history. Such a case where a change in average is largely caused by a shift of records from the peak to the tail, is strong evidence for multiple underlying distributions. We interpret the peak to be related to cows with four uninfected quarters.

The statistical distribution of cow-SCC observation was best described with a mixture of four normal (N) distributions and one exponential (E) distribution. Analysis of a second independent dataset with 1,546,570 records on 58,070 cows yielded similar parameters of these five distributions. This is evidence that datasets by cow, sire, herd, parity, stage of lactation, etc. may be characterized by the percentage of records in each underlying distribution (Table 1).

<table>
<thead>
<tr>
<th>Subset</th>
<th>Subset level</th>
<th>pN1</th>
<th>pN2</th>
<th>pN3</th>
<th>pN4</th>
<th>pE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous record</td>
<td>Low (&lt; 100,000 cells/ml)</td>
<td>0.33</td>
<td>0.34</td>
<td>0.21</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>High (≥ 100,000 cells/ml)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.23</td>
<td>0.34</td>
<td>0.40</td>
</tr>
<tr>
<td>Previous 2 records</td>
<td>Low – low</td>
<td>0.35</td>
<td>0.38</td>
<td>0.20</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Low – high</td>
<td>0.06</td>
<td>0.10</td>
<td>0.42</td>
<td>0.22</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>High – low</td>
<td>0.09</td>
<td>0.20</td>
<td>0.40</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>High – high</td>
<td>0.00</td>
<td>0.00</td>
<td>0.09</td>
<td>0.43</td>
<td>0.48</td>
</tr>
<tr>
<td>Parity</td>
<td>1</td>
<td>0.26</td>
<td>0.32</td>
<td>0.23</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.22</td>
<td>0.26</td>
<td>0.26</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.17</td>
<td>0.22</td>
<td>0.25</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.13</td>
<td>0.16</td>
<td>0.20</td>
<td>0.18</td>
<td>0.33</td>
</tr>
</tbody>
</table>

We interpret N1 as uninfected cows, N2 as cows recovering from an infection, N3 as cows with a non-persistent infection and N4 and E as cows with persistent infections. The commonly reported parity effect on SCC appears to be an effect of persistent infections increasing with age.

On this basis, we defined several new SCC traits. ‘Suspected’ is the probability that at least one SCC in a lactation originated from N3, N4 or E. ‘Seriousness’ is the percentage of SCC in a lactation that originated from N3, N4 and E. ‘Severity’ is the probability that at least one SCC in a lactation originated from N4 or E. ‘Duration’ is the length of the longest streak of SCC originating from N3, N4 or E. Estimating the percentages of records in each underlying distribution for a herd may also be a useful tool for veterinary advice.
Automatic detection of mastitis using sensors and Internet: a demonstration project.

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Introduction
Increasing herd sizes and the more frequent use of automatic milking machines increase the need for automatic detection of mastitis (Hogeveen and Ouweltjes, 2003). Automatic detection may be based on in-line sensors that record electronic conductivity, yield and temperature at milking. Although these sensors have been commercially available for several years, the uptake of this technology by dairy farmers has been limited. One of the reasons may be the low specificity of the reported alerts which are based on simple algorithms. However, the use of more advanced statistical techniques and the simultaneous use of multiple types of sensor data can significantly improve the quality of the alerts (De Mol and Woldt, 2001). Further improvements may also include the use of information from a central database and the presentation of the alerts in more user-friendly reports. Hence, the aim of this demonstration project was to implement an Internet-based application that combined multiple types of sensor data and used advanced techniques to produce mastitis alerts.

Material and methods
In an experiment, two Dutch dairy herds (110 and 120 cows) recorded electric conductivity (at quarter level), milk yield and milk temperature (June-August 2006). These sensor data were transferred to the central NRS database using Simple Object Access Protocol (SOAP) services. Mastitis alerts were produced at NRS in two steps (De Mol and Woldt, 2001). First, a time-series model used the sensor data to generate alerts at cow level. Secondly, a fuzzy-logic algorithm classified the generated alerts into true positive (TP) or false positive (FP) using additional cow data from the NRS database. The final alerts were presented to the farmers via a web-based management system. For every alert, the severity (weak, moderate and severe) of the underlying effects which triggered the alert (e.g., increased conductivity) were presented. After the experiment, the farmers discussed the results and follow-up actions at a workshop.

Results
On average, the daily number of mastitis alerts was 0.6 and 0.9 at the two herds, which was much lower than with their local detection systems based on electric conductivity only. Both farmers reported no false-negative alerts (all treatments of clinical mastitis were detected) and a low number of false-positive alerts (only a few alerts did not correspond to a treatment or increased somatic cell count levels). At the workshop they both indicated that the mastitis detection system improved monitoring and management of mastitis.

Discussion and conclusions
The main aim of this study was to demonstrate the technical and practical feasibility of an Internet-based application to produce reliable mastitis alerts. At the two participating herds, the implemented system showed a large practical relevance as it presented the farmers more useful information. In new studies, a more quantitative validation of the produced alerts based on more frequent somatic cell count observations and bacteriological analyses is suggested. Such analyses can also help to formulate more specific management actions in relation to the severity of the mastitis alerts.

In this study, sensor data were processed centrally. The advantage of central processing is that it capitalises on centrally stored information. Central data processing enables alerts being integrated into other herd-management reports including benchmarking and, in the long term the use for breeding purposes. Further synergy opportunities exist when submitted sensor data are also used to produce alerts for other health traits such as oestrus and lameness and/or when more advanced in-line sensors for SCS and clinical mastitis (Mottram et al., 2007) are included.

References
Improvement of mastitis detection, pathogen diagnosis and online decision support around treatment for automatic milking systems using additional non-automatic milking system data

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In automatic milking systems (AMS), clinical mastitis (CM) is currently detected by sensor measurements only. With these systems, the number of false-positive warnings for CM is currently too high. Additionally, the current systems can not predict the involved pathogen for CM. It is expected that further improvement of the detection of CM and the involved pathogen is possible by taking into account additional cow data, for instance, data on monthly somatic cell count (SCC) measurements and CM history.

In this project, models will be developed, based on additional cow data that could be combined with AMS data to improve the detection of CM. These models need to determine the risk of CM for individual cows. Additionally, for cows having CM, models will be developed that can predict the involved pathogen. For this part of the project, a dataset containing more than 8,000 CM cases with information on the involved pathogen was used.

In the first part of this project, a multivariate logistic regression model was developed including several cow-risk factors (non-AMS). The following cow-risk factors were significantly associated with the risk on CM: parity, month in lactation, season of the year, geometric mean SCC of previous lactation, SCC in the previous month, the accumulated number of CM cases in the previous month of the lactation and the accumulated number of CM cases in the month of lactation before the previous month of lactation. With the developed logistic regression model it is possible to determine the risk of CM for individual cows. This logistic regression model needs to be updated with daily information of the sensor measurements generated by the AMS. It became clear that for this purpose logistic regression models have several disadvantages. Logistic regression is not a flexible tool when new information becomes available and moreover, it can not handle missing values.

In the second part of the project, CM and the associated significant cow-risk factors will be analysed with Bayesian Networks to determine the risk of CM for individual cows. This statistical method is more flexible to include new information of the cow. This part of the project also includes the prediction of the involved pathogen. Naive Bayes classifiers are part of the Bayesian Networks and especially valuable for classification tasks. Therefore, these classifiers are currently used for the prediction of the involved pathogen for CM cases. For these analyses information on cow-risk factors and clinical signs of the CM case are used. First results show an accuracy of approximately 50% to distinguish between streptococci, staphylococci and *E. coli*.

Results of prediction models for the involved pathogen will be used for the third part of the project. In that part of the project, an online decision support model around the treatment of CM cases will be developed. In these decision support models, the farmer will be supported in the decision for different treatment options. Currently, work on this project has started.
Inline Somatic Cell Count improves the detection of clinical mastitis in an automatic milking system.

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Current automatic milking (AM) systems generate mastitis alerts by using sensor information, with the most common sensor being the electrical conductivity (EC). However, a common complaint by farmers using AM is that there are too many false positive alerts on these lists. Somatic cell count (SCC) is also measured off-line as an indicator for udder health status, but is measured infrequently. Recently, a new sensor has been developed, estimating SCC inline (Whyte et al., 2000). This study investigated the potential of using this sensor for clinical mastitis detection. Data was sourced from a research farm in New-Zealand, and included 194 cows milked by 2 AM systems during a period from the 1st of July 2006 to 1st of May 2007. Data included EC, inline SCC of composite milk per cow (ISCC), periodic laboratory testing of SCC in composite milk per cow and antibiotic treatment records of clinical mastitis.

A frequency table was constructed (Table 1) to see whether ISCC measurements were in agreement with fortnightly laboratory-determined SCC (FSCC). SCC measurements were divided into 6 categories. Table 1 shows that 86% of all milkings with a FSCC lower than 200,000 cells/ml also had an ISCC lower than 200,000 cells/ml; 84% of all milkings with a FSCC higher than 200,000 cells/ml also showed an ISCC higher than 200,000 cells/ml. Pearson correlation coefficients between normalized (log base 10) values of ISCC and FSCC were 0.24 for FSCC values lower than 200,000 cells/ml and 0.83 for FSCC values higher than 200,000 cell/ml.

Table 1. Number of cow milkings falling into a specific SCC category when determined by inline SCC or fortnightly SCC measurements

<table>
<thead>
<tr>
<th>In-line SCC (x1,000 cells/ml)</th>
<th>≤50</th>
<th>50 - ≤100</th>
<th>100 - ≤200</th>
<th>200 - ≤500</th>
<th>500 - ≤1,000</th>
<th>&gt;1,000</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50</td>
<td>31</td>
<td>108</td>
<td>68</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>214</td>
</tr>
<tr>
<td>50 - ≤100</td>
<td>18</td>
<td>48</td>
<td>77</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>153</td>
</tr>
<tr>
<td>100 - ≤200</td>
<td>13</td>
<td>36</td>
<td>114</td>
<td>44</td>
<td>1</td>
<td>2</td>
<td>210</td>
</tr>
<tr>
<td>200 - ≤500</td>
<td>12</td>
<td>16</td>
<td>43</td>
<td>143</td>
<td>23</td>
<td>2</td>
<td>239</td>
</tr>
<tr>
<td>500 - ≤1,000</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>31</td>
<td>57</td>
<td>11</td>
<td>111</td>
</tr>
<tr>
<td>&gt;1,000</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>75</td>
<td>78</td>
</tr>
<tr>
<td>Totals</td>
<td>79</td>
<td>213</td>
<td>307</td>
<td>236</td>
<td>89</td>
<td>81</td>
<td>1,005</td>
</tr>
</tbody>
</table>

Using EC only as a detection tool for clinical mastitis and setting the parameters to achieve a sensitivity (SN) of 80%, the positive predictive value (PV+) was 9.4% and the false positive attentions per 1,000 milkings (FP1000) was 7.8%. Figures using ISCC only were 11.3% and 6.1%. A fuzzy logic algorithm was developed that combined EC and ISCC information, and again parameters were set such that a SN of 80% was reached. Using the combination of sensors, the PV+ increased to 15.6% and the FP1000 decreased to 4.4%.

These results suggest that measuring composite inline SCC makes a worthwhile contribution to an automatic sensing system for the detection of clinical mastitis.
Detection of mastitis and abnormal milk with automatic milking systems

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Automatic milking (AM) has proven to be a viable alternative to traditional milking systems, providing dairy farmers with more flexible working hours without negatively affecting cow welfare. However, obstacles related to the detection of abnormal milk and clinical mastitis remain to be solved to improve adoption. While automated separation of visually abnormal milk with AM systems will be required by legislation in the near future, this has not yet been implemented. Separating abnormal milk automatically requires a reasonable detection rate combined with few false positives to limit the amount of milk that is discarded unnecessarily. Currently used AM systems are able to detect a reasonable proportion of clinical mastitis cases, but only at the expense of predicting many normal milkings as having mastitis, which requires much time and effort from the dairy farmer to separate false positives from real cases. Once a case of clinical mastitis has been detected, the dairy farmer needs support to determine a proper treatment, often involving antibiotics. Patterns in the milk quality data measured with AM systems are expected to help determine the type of pathogen causing the mastitis, although this has not yet been investigated extensively. The detection, diagnosis, and treatment of mastitis might be further enhanced through the use of additional, non-AM system, data such as somatic cell count, mastitis history, pathogen prevalence, and antibiotics effectiveness, which are available at many farms.

This project aims to enhance AM systems with new or improved data processing modules that deal with abnormal milk and clinical mastitis. Development of these modules can be grouped into two related areas of investigation. The first area covers the detection of abnormal milk, mastitis, and mastitis causing pathogens based on sensor data from the AM system. Data mining techniques will be used to develop detection models from large amounts of data acquired from commercial farms. Improved classification models for mastitis or abnormal milk may be created by combining data from multiple sensors (e.g., electrical conductivity (EC), colour, and milk flow) as well as through proper pre-processing of the sensor data patterns. Furthermore, assessing the value of additional sensors, like inline somatic cell count, is part of the project (see abstract Kamphuis et al.). The second area of investigation is intended to improve the detection of mastitis and mastitis pathogens and to develop mastitis treatment plans by taking into account non-AM system data. (For more information on the second part of the project see the abstract of Steeneveld et al.)

At this moment, 9 farms with a total of 12 AM systems (Lely Astronaut) are involved in the project. Sensor data are automatically logged and farmers are asked to record if there were clinical signs of mastitis or not for each cow on the daily mastitis attention list. For each treated case of mastitis, the farmer is asked to take a milk sample of the infected quarter for bacteriological culturing (BC). The quarter milking before the start of a mastitis treatment will be labelled as having mastitis. Quarter milkings from cows without clinical mastitis during their lactation and milkings labelled by the farmer as having no clinical signs will be used as negative cases for mastitis and pathogen detection.

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Milking and udder health projects

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Preliminary results of 3 projects of UGCN.

Beest 2: Milking time test to improve milking (ASG, GD) (Half way)

Farms were selected on bulk tank cell count of 2005: low (<101), mid (200-250), high (>350). On 200 farms a milking time test is performed and a profile of the farm is made. Farms received written advice. On average (40 farms, Figure) 50% of the advices is followed up. Milk recording data of the farms is followed from 1 year before till 1 year after the milking time test and control farms are included. Farmers scored percentage of clinical mastitis during visit: 21, 25 and 25%. The first results after 6 and 12 months show a lower percentage. Some farms encountered Blue Tongue and increased mastitis incidence. Analysis of data will start after July 2008.

Beest 3: Measurement of detachment level of automatic cluster removal equipment (ACRs) (Finished)

Timely detachment of the cluster at the end of milking will promote udder health and milking efficiency. A method and device to measure detachment level is developed. Automatic detachment of the cluster at end of milking is initiated by an electronic milk meter or indicator (electronic/mechanical). Problems in measurement involve variation in milk flow due to milk slugs in the long milk tube and variation in response of the metering device.

Cluster removal should be initiated by a dedicated sensor because milk meters are designed to do a correct measurement of the amount of milk and are slow and inaccurate for (low) milk flow measurements at the end of the milking process.

Especially milk meters with a buffer are the most inaccurate cluster removal indicators.

Advice on detachment to the farmers can be given on how to adjust the detachment settings of milk meters and sensors, maintenance and cleaning is important for best results. Moreover visual inspection and milking technique is important. Measurement of detachment level is advised after installation and as an option during yearly dry testing.

Beest 9: Risk factors for udder health on farms milking with an automatic milking system (ASG, FD) (Just started)

For advice on udder health in farms with automatic milking systems sound knowledge is still limited. In this project risk factors and management measures will be delivered from input of expert teams and risk assessment on 150 farms. The expert teams consist of veterinarians, researchers, manufacturer and farmers., Farms will be visited by veterinarian students using an enquiry list originating from the expert meetings.
Costs of mastitis and efficiency of management measures

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Introduction
To improve udder health, which is considered one of the most costly diseases in the dairy sector, farmers need to apply available knowledge. There are two main economic factors that motivate farmers to improve udder health, first a penalty- or bonus system and secondly insight in the costs and benefits of different management measures. The latter will be discussed here.

Economic losses
The economic damage of mastitis consists of several categories: milk production losses, drugs, discarded milk, veterinarian, labour, milkcomposition, culling, and increases susceptibility for other diseases. Milk production losses account for the biggest part in the financial losses of mastitis and occur in both, clinical and subclinical mastitis. In the past calculations for clinical mastitis have been made. These calculations, however, are very rough and thus not useful for farm specific calculations. New calculation-methods, including variation, were made. With this method, the economic losses of a clinical case of mastitis in a default situation were calculated at €210, varying from €164 to €235 depending on the month of lactation. The total economic losses of mastitis (subclinical and clinical) per cow present in a default situation varies between €65 and €182 per cow per year depending on BMSCC.

Estimations by farmers
Most farmers (72%) expected their economic losses to be lower than the costs calculated, using their farm-specific information. Underestimating the economic losses of mastitis can be regarded as a general problem in the dairy sector. The average economic losses assessed by the farmers were €78 per cow per year, with a large variation, €17 to €198 per cow per year. Although the average assessment of the farmers of the different cost factors is close to the default value, there is much variation. To improve the adoption rate of management practices to improve udder health, it is important to quantify the economic losses of mastitis.

Udder health management
Different management measures to improve udder health are available. Some of the most frequently recommended mastitis control practices are hygienic washing and drying of udders before milking, regular milking machine maintenance, teat dipping/spraying after milking, appropriate treatment of clinical cases, culling of cows with chronic mastitis and milking infected cows last. Every farmer has his own ideas about the different measures and because of the lack of information about the efficiency of the measures; decisions are often made intuitively. Behavior of the farmer is often not consequent with the economical advice. The valuing of money is not in every situation the same. When a farmer has a problem he wants to get rid off, most of the times he want to spend more money then when he can prevent the problem (stable vs. unstable situation). Another difference exists between the valuing of different kind of investments like long term expenses, short term expenses, labour, and changing of routines. Because of lack of knowledge on the efficiency of management measures, sometimes contradictory advices are given. Thus, the adoption rate and efficiency of implementation of advices given vary widely.
To our opinion it is necessary to develop a farm specific calculation-method of the different management measures, based on available knowledge on efficiency, costs and the farmers valuation. With such a tool, farmers can make well-funded decisions on udder health management.
Bioeconomic modeling of Bovine mastitis

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† Business Economics Group, Wageningen University

In the first phase, a review was conducted on published peer reviewed papers since 1990 related to the economics of bovine mastitis and mastitis management in dairy herd. The review revealed an average loss of €280 per case of clinical mastitis (CM) and an average loss due to SCM of €102 per case. It also showed a wide variation between the estimations of the different studies, which could be explained due to different country regulations, market prices, analytical methodologies, and cost factors included in the analysis.

In the second phase, the milk, fat, and protein losses due to new SCM were estimated based on a random regression test-day model (RRTM). A cow was considered to have a new case of subclinical mastitis if the somatic cell count (SCC) of the previous test-day was < 50,000 cells/ml and SCC of the current test-day was > 100,000 cells/ml. A primiparous cow with SCC 200,000 cells/ml lost 0.41 kg milk, 4.5 g fat and 10 g protein per day. A multiparous cow with the same SCC lost 0.6 kg milk, 10 g fat and 13 g protein per day during the new SCM episode.

Currently, a bioeconomic model of mastitis is being developed including the dynamics of mastitis pathogens in a Dutch dairy herd in a year with 2 weeks time intervals. The model simulated the 4 predominant mastitis pathogens (S. aureus, S. uberis, S. dysgalactiae, and E. coli) separately and independently. The dynamics of infection of the first 3 pathogens were explained by the transmission rates (β) of these contagious infections between cows. Due to the environmental route of E. coli mastitis, a Greenwood model was developed to explain the dynamics of infection based on the incidence rate. A cow was assumed to be either susceptible or infectious of either of CM and SCM. A susceptible cow can become infectious based on the probability of infection calculated from β, and the number of infectious and susceptible cows. A CM cow may recover based on the probability of recovery of 3 days treatment and therefore become susceptible, may persist as a SCM case then flare up as CM in the next period, or may persist as SCM (remission case). A SCM cow may spontaneously recover and become susceptible, may flare up as CM, or persist as SCM case. The model simulated a herd with 100 cows in a quota situation. Milk yield, calving interval, parity and calving season were assigned based on random distributions. Lactation stage was assigned based on the calving season and the calving interval. Milk production was modeled based on the Wood’s lactation curve. A SCC between 100,000 and 600,000 cells/ml was assigned to the SCM infections. A SCC of 750,000 cells/ml was assigned to CM infections. Accordingly the bulk tank SCC (BTSCC) and the geometric mean of the BTSCC were calculated based on the 2 weeks intervals. A new case of CM was considered when clinically infected at each time period. A new case of SCM was considered when infected after a healthy time period. Production loss of CM and SCM mastitis were included based on literature estimates. Each pathogen model was iterated 1000 times for a one year period. Validation of the model was conducted based on methods obtained from literature. These methods are rationalism, trace back, face validity and syntax debagging by an expert. The model is currently in the phase of validation and therefore the results are not available.
The Dutch Quality System for Milking Machine Maintenance

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2-Stichting Kwaliteitszorg Onderhoud Melkinstallaties (KOM), Dronten, the Netherlands

Introduction
In the eighties and nineties ISO standards were developed for milking machines. In the Netherlands these standards were implemented by ASG in a Quality System for the maintenance of milking machines. Later on an independent organisation KOM (Kwaliteitszorg Onderhoud Melkinstallaties). KOM has set up several actions and procedures to supervise and to control the quality system. The system guarantees the farmer that the maintenance of his milking machine and the necessary accuracy checks of milk meters and jars are performed well against minimal costs. The system has been incorporated in the total quality management systems for dairy producers within the Dutch dairies.

The KOM Quality System
KOM has developed several activities within the KOM quality system. These activities and the procedures are recorded in the KOM guidelines (KOM, 1999):

- Registration and evaluation of all test reports made by the technicians including reports on the accuracy of milk meters and jars,
- Annual control and calibration of the test equipment used by technicians,
- Performing random checks on the ‘quality of work’ of the technician including milk meters and jars,
- Certification of (new) technicians,
- Development of standard reports (MAR) and tests (based on ISO),
- Studies on the relation between milking machines and milk quality,
- Development of guidelines for new areas, like automatic milking systems.

All Dutch milking machines are checked and maintained annually by qualified technicians. The test results are recorded in a standard test report, which is equal for all manufacturers. A copy of the report is handed over to the farmer, another copy is sent to KOM. The reports are registered per technician and evaluated at random using an evaluation protocol. The results are discussed with each technician once a year. At Waiboerhoeve experimental station, the research facility of the Animal Sciences Group of Wageningen UR, a training and test centre was established. This centre has a special test installation suited to test and calibrate test equipment.

According to the requirements of KOM, technicians should be well qualified. KOM together with the Animal Sciences Group of Wageningen UR, has set up a special education program for milking machine technicians resulting in a special certificate. Over 350 technicians joined these courses and approximately 85% succeeded and obtained a certificate, so they are allowed to test milking machines within the KOM system. KOM performs random checks on farms to evaluate the quality of work by the technicians. If the technician is not doing a good job, KOM may decide to withdraw his certificate, so that he is not longer allowed to test milking machines.

Joining an annual retraining course including the accuracy check of the test equipment, is a prerequisite for keeping the certificate. More detailed information is available at the authors- contact kees.dekoning@wur.nl or harm.wemmenhove@wur.nl
Quarter-controlled milking

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Genetic progress, improved management and feeding, and higher milking frequencies are causing a strong increase in the daily milk yields per cow. As a consequence, the duration of milk removal increased drastically. More mechanical stress is exerted on the cows’ teats and udder, which is reflected by harmful effects on teat sphincters. Severe abnormalities in sphincters are often associated with mastitis. In current milking practice, often only half of the machine-on time is needed for removing more than three quarters of the available milk. The large inefficient parts in the milk removal process might also lead to a higher somatic cell count (SCC) in the milk and occasionally result in mastitis.

It is important to realize that the quarters of the udder are four separate compartments. Milk in a quarter can be divided in two categories: the alveolar milk stored in the alveoli and small ducts, and the cisternal milk, stored in the larger ducts of the udder and in the teat cistern. Mechanical stimulation of udder and teats causes the release of the hormone oxytocin in the blood. This release results in the milk letdown reflex, whereby milk from the alveoli is pressed into the ducts and cisterns of the udder. The action of the milking unit removes the milk from the teat. For efficient milk removal, information about the amount of available milk in the teat cistern is needed. Four main phases in the availability of milk during milking can be distinguished. In the first phase (P1), milk ejection from the alveoli has not started yet. Milk removed from the udder is cisternal milk that has leaked from the alveoli during the interval since last milking. The duration of this first phase is normally less than 90 s. In the second phase (P2), the flow rate of milk removed from the udder and teat cistern is at least equal to the flow rate of ejected milk from the alveoli. In the third phase (P3), the flow rate of milk ejected from the alveoli is smaller than the flow rate of the removed milk. In the fourth phase (P4), the flow of milk from the alveoli has stopped and the available amount in the udder and teat cistern approaches the zero level. The effects of a number of factors on these milk removal parameters are shown in table 1.

Table 1. Effects of prep lag duration and pulsation settings on milk yield, bimodality and milk removal parameters.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Milk yield (kg)</th>
<th>Bimodality (%)</th>
<th>P1 (s)</th>
<th>P2 (s)</th>
<th>P3 (s)</th>
<th>Duration quarter milk milking (s)</th>
<th>P4 (s)</th>
<th>Duration udder milk milking (s)</th>
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<td><strong>Prep lag duration</strong></td>
<td></td>
<td></td>
<td></td>
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<td>&lt; 10 s</td>
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<td>340</td>
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<td>380</td>
</tr>
</tbody>
</table>

It could be concluded that milk removal efficiency will be improved when after attachment more cisternal milk is available (e.g. after a longer prep lag duration) and quarter switch-off is implemented. The effect of different pulsation settings on milk removal is mainly expressed during the second phase (P2) of the milking process. In the other phases too aggressive pulsation settings might have harmful effects on teat and udder tissue. Future milking machine technology should be able to adapt the machine settings to individual quarters and during milking to the availability of cisternal milk in order to reach a high milking capacity without negative effect for udder health.
Udder health and farm management on organic dairy farms without antibiotics

Gidi Smolders

Animal Sciences Group of WUR

At nine dairy farms (four with suckling the calves) aiming at zero antibiotic use, management and medicine use were recorded during a one year period by a questionnaire and individual farm visits. Main items were in with occasions antibiotics are still needed, are antibiotics replaced by other medicine, are treatments postponed, is health and welfare secured and is management changed. Cows on ab-free farms were as old as the average organic cow (57 months) and 305 days yield was 5900 kg milk versus 7100 kg for the average organic dairy cow. Selection criteria for breeding bulls changed from production traits to health traits (mastitis index, longevity). The main problem was keeping udder health at an acceptable level. Herbs and homeopathy replaced antibiotics in the beginning of the ab-free period, later on medicine use reduced. A high somatic cell count was easily accepted as well as three teat cows.

Hardly any preventive measures were taken: more cows are prepared with one cloth, cows are not tested with formilking, diseased cows are not milked separately and cows can lay down immediately after milking. Four farms rinse the teat cups with hot or cold water after a diseased cow has been milked and three of the farms post dip with an iodine solution. Farmers take action if there is a serious risk of a too high somatic cell count in the bulk milk. In case of clinical mastitis a variety of treatments are practised with antibiotics as ultimate remedy. Farmers state that treatments sometimes are postponed to long to offer the animal the opportunity to spontaneous cure. Some quarters are lost even after treatment. Choices are difficult: offer the cow the opportunity to self healing or administer antibiotics and sell the cow afterwards. In some cases antibiotics are used as dry cow therapy. At four farms non antibiotics are used for udder health, these farms practice good milking, homeopathy, eco therapy, reflection, drying off the affected quarter and/or selling the cow. The percentage of high somatic cell count cows exceeds the average conventional percentage. The best farm scores only 12% cows exceeding 250.000 cells/ml milk but there is also a farm with over 40% high cell count cows constantly. The farmers have no idée which pathogens causes the possible mastitis problems. The dry period gives spontaneously healing of somatic cell count problems; the number of low cell count cows increases during the dry period. At the ab-free farms at drying off 64% of cows are low cell count cows while cows in a research on conventional farms score 70% with antibiotic dry cow therapy. After calving the percentages are respectively 68 and 84%, with a great variety between farms. It shows that not only antibiotics play a roll in the curing effect of the dry period. It can be concluded that udder health should have constant attention and involves the whole management on the farm, including preventive measures and adequate feeding with minerals and trace elements. Antibiotics can not be faded completely.

In 2007 30 different herbs are tested to find out if and at what dilution the growth of Staph. aureus and Strep. Uberus is limited. In milk Cinnamomum verum, Hulumus lupulus, Thymus vulgaris. Origanum vulgaris and a medium chain fatty acid did prevent bacterial growth. The promising extracts will be tested in cows next month on possible unexpected reactions. In 2008 extracts will be tested as medicine to cure (sub) clinical mastitis. On 50 practical dairy farms udder health and treatments are part of research into measurements influencing resistance in cows. The first aim of the research is to see if there are differences in resistance between groups of farms: high and low percentage of high somatic cell count cows in the herd, young and old herds, farms with calves suckling and farms with a high health status. It is striking that the longer farms are organic, the more complementary medicine is used.