

Mastitis Newsletter

26/
2005



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Mastitis Newsletter 26

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Foreword

Mastitis Newsletter aims mainly at disseminating succinct information on the work, plans and achievements of the IDF Standing Committee on Animal health, but also includes information available from other sources such as the National Mastitis Council (NMC) in the USA. The IDF Standing Committee and the NMC have a close working relationship.

Mastitis Newsletter does not intend to review systematically the vast literature in the field of mastitis nor does it claim to report on all significant developments in the field.

Information given and statements made in Mastitis Newsletter do not commit IDF. They can be reproduced, with indication of source.

Contributions dealing with items of general interest would be welcome for consideration for inclusion in future issues.

Mastitis Newsletter is available from International Dairy Federation (IDF), Head Office, 80 Boulevard A Reyers, B-1030 Brussels (Belgium). It is produced in English only and it is expected to be of interest to persons studying the many aspects of mastitis, to veterinarians, research institutes, students, etc.

Current membership of the IDF Standing Committee on Animal health

Chair: Laura Kulkas (FI) **Deputy chair:** Alfonso Zecconi (IT)

K Aagaard (DK), M Beguin (FR), Y Botton (BE), P Brightling (AU), R M Bruckmaier (DE), Chr Burvenich (BE), C Chan (AU), M Chriél (DK), M T Collins (US), R Condrón (AU), N Das (US), L De Meulemeester (BE), T Ekman (SE), L J Erasmus (ZA), A Founta (GR), G Gradillas (ES), J Hamann (DE), J E Hillerton (GB), J S Hogan (US), H Hogeveen (NL), S Ijkema (NL), H Itabashi (JP), O R Jepsen (DK), M Klopčič (SI), K Knappstein (DE), P Latham (GB), A Lesser (DE), Yaohua Lu (CN), C McCrindle (ZA), J McQueen (AU), W Meaney (IE), G Mein (AU), A Michel (ZA), I Michelutti (FR), A Moncada Jiménez (MX), V Myllys (FI), S Neddunchellian (IN), K O'Farrell (IE), S Orlandini (IT), O Østerås (NO), N N Pandey (IN), L H Pedersen (DK), K Plym Forshell (NO), B Poutrel (FR), P Rainard (FR), S Ramantanis (GR), P Ruegg (US), A Saran (IL), M Schaellibaum (CH), W Schaeren (CH), G K Sharma (IN), R Silber (AT), D K Singh (IN), B Sonck (BE), W Sykes (AU), A Syrris (GR), A Touratier (FR), B C Varshney (IN), G Verkerk (NZ), J Vignal (CH), J L Villicaña (MX), E Vindel (FR), M Wetzstein (CA), E X Frangiadaki (GR), I Zarzouras (GR), O Zorko (SI).

Note of the editor

The mastitis newsletter: IDF continues to work on mastitis.

Henk Hogeveen (NL)

*Faculty of Veterinary Medicine, Utrecht, the Netherlands
Wageningen University, Wageningen, the Netherlands*

The International Dairy Federation (IDF) represents a unique body of knowledge on dairy matters. With 41 member countries, representing 73 % of the total milk production, IDF's events are a very effective way of sharing knowledge. They bring together the world's top dairy experts and provide a forum for exchange of ideas and experience. Mastitis is a long-standing focus field in IDF, as is the mastitis newsletter. Not intending to be a scientific or a trade journal, the newsletter aims at giving a view on current issues relating to mastitis on a world-wide basis.

We are glad that another issue of the Newsletter is lying or on the screen in front of you. As a first, since IDF is going to be more and more digital, this newsletter is sent to you by e-mail.

It has been some time since the previous mastitis newsletter. This does not mean that nothing happens in the mastitis world. On the contrary there is a lot of action in the mastitis world: just take a look at the Events & meetings section of this newsletter. Mastitis remains an important disease in dairy cattle and is still regarded as the disease with the greatest economic damage for the dairy farmer. Moreover, when demanding consumers require milk products of high quality, the effects of mastitis can be seen in the image of milk and milk products as such. From this point of view it is not remarkable that almost 350 research abstracts were submitted for the 4th IDF International Mastitis Conference, to be held in Maastricht this year. In this issue of the Newsletter you can read more about it.

I want to thank all contributors for their efforts to keep the Mastitis Newsletter interesting for you, our reader. And to all of you, if you have announcements, meeting reports, mastitis information or short research communications, I invite you to send them to me (henk.hogeveen@wur.nl) so we can publish them in the IDF Mastitis Newsletter in the future.

IDF Standing Committee of Animal Health

November 2003 - April 2005

Laura Kulkas

laura.kulkas@valio.fi

Chair of the IDF Standing Committee on Animal Health

The Standing Committee of Animal Health (SCAH) of the International Dairy Federation (IDF) was reorganized in November 2003. Laura Kulkas, Finland, was named chair and Alfonso Zecconi, Italy, was named deputy chair. Three Action Teams (AT) were formed:

1. Action Team of Mastitis, leader Joe Hogan, USA
2. Action Team of Infectious Diseases, leader Karsten Aagaard, Denmark
3. Action Team of Production Diseases, leader Torkel Ekman, Sweden

The planned fourth action team for emerging country issues was not set up. A process is going on within the IDF regarding the need of the emerging countries to get help to develop their milk production. This could entail setting up a "help network" and / or "training the trainers" schooling events.

Action Team on Mastitis has met twice in the USA in conjunction with the National Mastitis Council seminars 2004 and 2005, and once in Melbourne in November 2004. A very interesting seminar on *Streptococcus uberis* was held in Melbourne. The work of the AT has mainly focused on a review of the development of antimicrobial resistance. Economic Consequences of Mastitis by Olav Österås has been accepted and published as Bulletin of IDF No. 294/2005.

Action Team on Infectious Diseases met in Cape Town in March 2004 and in Melbourne November in 2004. Several cattle diseases are of interest to the dairy industry. BSE and paratuberculosis continue to be of main concern. Also FMD and tuberculosis have been on the agenda during recent years. A very interesting seminar on tuberculosis was arranged by the AT in Melbourne. The cooperation with the FAO and especially the World Organisation for Animal Health (OIE) has increased remarkably in the recent years. An IDF Working Party has been established to handle the large range of questions arising in the cooperation with the OIE. There is for example cooperation in the harmonization of the animal health requirements for export certification.

Action Team on Production Diseases met in the Netherlands in March 2004 in conjunction with the Conference on Automatic Milking. The main issues to be considered are lameness, metabolic disorders, fertility disorders and problems caused by parasites. A document on Lameness was prepared by the AT based on T. Manske's (SE) Ph.D. thesis. It was introduced to the SCAH in Melbourne in November 2004.

Members of the SCAH have attended various meetings or seminars during the last 18 months. Many members participated in the publishing of the IDF/FAO Guide to Good Dairy Farming Practice launched in Cape Town, South Africa in March 2004. Members have also attended several OIE meetings including a seminar on animal welfare in Paris in 2004 as well as the yearly National Mastitis Council seminars in the USA.

The next event will be the Mastitis Seminar in Maastricht, the Netherlands, 12-15th June 2005 with the SCAH meeting to be held on 12th June. The next AT on Infectious Diseases meeting will be held in conjunction with the International Colloquium of Paratuberculosis in Copenhagen, 14-18th of August 2005. The SCAH will meet again in conjunction with the IDF World Dairy Summit in Vancouver September 2005. The SCAH is involved in the planning of an Animal Health Conference at the IDF World Dairy Congress Shanghai 2006.

Revision of the Analytical Standard on Somatic Cell Counting in Milk

Harrie van den Bijgaard¹, Silvia Orlandini², Christian Baumgartner³

Among the expanding variety of tools for diagnosing mastitis, somatic cell counting is still one of the most indicative parameters for udder health status. On-going development in counting technology has resulted in the routine application of high capacity flow cytometric counters with much improved performance in central milk testing laboratories. Furthermore, attractive solutions for small scale somatic cell counting are finding their way to the relevant spots in the whole dairy chain.

As with all analytical work, standardization and harmonization are essential for the comparability of results between different places and over time and hence for the beneficial use of data. Under the aegis of the IDF Standing Committee on Quality Assurance, Statistics of Analytical Data and Sampling, the ISO/IDF Joint Action Team on Automated Methods is revising International Standard ISO 13 366|IDF 148. The new analytical standard on the enumeration of somatic cells will consist of two parts. Part 1 will contain an improved description for the execution of direct microscopic somatic cell counting (DMSCC), which is proposed to be retained as the reference method. Part 2 will provide guidance on the operation of routinely operated fluoro-opto-electronic counters. The scope of both parts will be expanded in order to include also somatic cell counting in sheep, goat and buffalo milk.

Special attention is given to overcome the remaining limitations of the reference method in its role as an anchor for the calibration of routine methods. For that purpose a reference system approach is under development wherein the use of reference materials with jointly established reference values and feed-back mechanisms will help to reduce variations and balance fluctuations in counting levels between laboratories and over time.

The Draft international standard (DIS) version of Part 2 will be circulated for comment to ISO Member Bodies and IDF National Committees during the first months of 2005.

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The Impact of Changing Dry Cow Strategies.

Elizabeth A Berry

Ph.D. thesis University of Warwick 2002

Dry cow antibiotic therapy of the lactating mammary gland of dairy cows aims to reduce the prevalence of mastitis, by eliminating existing infections and reducing the number of new infections acquired, during the non-lactating period and is a key component of the Five Point mastitis control plan.

Administering dry cow therapy to all cows is criticised as indiscriminate use of antibiotics that could lead to the development of antibiotic resistance and antibiotic residues. No evidence exists to support these hypotheses although changes in antibiotic resistance patterns have been noted in other animal production sectors.

Alternatives to dry cow antibiotic therapy were examined for their effects on the incidence of new intramammary infections and their impact on milk production and quality. Two, selective dry cow trials compared a negative control group with either dry cow antibiotic or an intramammary teat seal. Both treatments significantly reduced the number of new infections compared with untreated cows, both at the cow and quarter level, for clinical infections during the dry period, infections at calving and clinical infections during the first trimester of lactation. The teat seal had no significant therapeutic effect against infections present at drying off.

Analysis for quarter interdependence (clustering) demonstrated an effect only in untreated cows in both trials. This indicates that susceptibility or resistance to infection exists at the cow level and that treatment should be at the cow level. Analysis confirmed that existing infections at drying off by *Corynebacterium* spp. or coagulase-negative staphylococci are risk factors, increasing susceptibility to other infections during the dry period.

Practical decision making on dry cow treatment strategy must also include economic evaluation as well as other risk factors. This work concluded that using dry cow antibiotic therapy was still the most cost effective strategy although this would be influenced by the relative prices of the products.

The International Symposium “Automatic Milking, a better understanding” Lelystad 2004.

A. Meijering (NL)

Animal Sciences Group, Wageningen UR, Lelystad, the Netherlands

The International Symposium on Automatic Milking, Lelystad March 24-26 2004, was attended by 340 participants from 24 countries. Many came from the Netherlands and neighbouring countries but others from as far as Oceania, Japan and North America. In a friendly atmosphere a total of 48 oral and about 80 poster presentations were given and discussed. Around 60% of the presentations originated from the EU research project on the implications of automatic milking. It is virtually impossible to summarize everything presented. Therefore, a number of rather personal, statement-like conclusions and queries have to suffice here. All information presented is available in the proceedings (<http://www.wageningenacademic.com/automaticmilking>).

It has become quite clear, at least in Europe, that farmers are presently emphasizing the economic and even more the social component of sustainability. Dairy farmers desire an appropriate income, less hours of labour and a larger share of social life. Options are to hire (cheap) labour, to start new co-operatives or automation. Where cheap labour is not available and tying up in a co-operative is not an option the automatic milking system comes into the picture.

The room for investment in automatic milking is determined by the value of labour savings or appreciation of spare time/freedom/ anticipated, augmented with the value of the expected additional milk yield and costs saved on investment in a conventional milking parlour, divided by the yearly costs of the automatic milking system(s). So where labour costs are high or more spare time is highly appreciated, automatic milking becomes an attractive alternative. In Europe, farmers adopting automation at present adopters are middle sized enterprises with high numbers of cows (50-100) and high herd yields per hand (>700.000 kg). Labour is thus under pressure. In North America however, the future may show a quite different pattern of adoption. Penetration of automatic milking may be slowed down by decreasing margins on milk, the inherent inflexibility of the AM-systems when increasing herd size and difficulties in combining automatic milking with grazing. With respect to the latter we may however learn a lot from New Zealand and Australia in the near future.

The attitude of press and society towards automation of milking can be characterized as indifferent to slightly favourable and is expected to remain so, unless automatic milking is connected with a product quality scandal or grazing of dairy cattle becomes a real political issue.

Automatic milking created pressure for a new definition of abnormal milk in the EU Hygiene Directive. Such a new definition, endorsed by experts, is now available. It will be a challenge for the industry to develop accurate sensors for in-line sorting of abnormal milk in conformity with this definition. Although milk quality requires attention during the transition period from conventional to automatic milking, in general no serious problems are encountered afterwards. The observed increase in free fatty acids demands more research. Moreover, the importance of farm hygiene, adequate udder and teat cleaning and accurate system cleaning should be stressed.

When changing to automatic milking, hardly any negative or positive effects on animal health and welfare are observed. Udder health, particularly in fresh cows, and claw health should be carefully monitored, specially when the herd is kept indoors; so should the intake of dry matter by lower ranking cows. Finally, for the sake of improved management support, the industry is challenged to realize a better integration between milking system and herd management software, to improve control functions of the milking system and to develop sensors for management support further.

The 4th IDF International Mastitis Conference

or: All there is to learn about mastitis

Henk Hogeveen

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Mastitis is a long-standing field of focus in IDF. Since 1975, every 10 years IDF has organized a world mastitis conference. This year, the 4th IDF International Mastitis Conference is organized by the Netherlands National Committee of IDF and will be held in Maastricht, the Netherlands from 12-15 June. The conference is regarded as the worldwide event allowing the exchange of fundamental and applied research results and knowledge in the field of mastitis and mastitis-related issues, such as milk quality and farm management.

The first announcement with regard to the Conference was sent out almost 2 years ago. A year later the call for papers was sent out. Researchers around the world were asked to send in their research findings. More than 340 abstracts were received. Based on this huge amount of (high quality) abstracts, the scientific committee has selected the 115 best abstracts for oral presentation. The other abstracts can be presented as a poster presentation. Besides these contributions, 13 keynote presenters were invited to present their unique perspective on the developments in mastitis research and control. The presentations are organized in 11 sessions:

- **Diagnosis of mastitis & Indicators for milk quality**

Definitions of mastitis have been an ever-lasting debate. This session deals with bacteriological diagnostics but also with the use of in-line sensors to detect mastitis. Somatic cell count has become an important aspect in mastitis control. Papers discussing somatic cell count and possible other mastitis indicators for milk quality and limits of somatic cell count are going to be presented in this session.

- **Economics of mastitis and mastitis management**

Mastitis is often referred to as the cattle disease that is economically the most important. This session deals with economic subjects with regard to mastitis and mastitis control.

- **Pathogenesis and Immunology**

Research on pathogenesis of mastitis and the immunology of ruminant mammary gland mechanism is going to be presented in this session

- **Milking technology**

Milking and milking technology are associated with mastitis occurrence. Papers regarding milking technology are going to be presented in this session.

- **Animal welfare issues related to mastitis**

Mastitis can be a painful disease and thus influences animal welfare. In this session papers on the relation between mastitis and animal welfare are presented.

- **Environmental control**

The environment has a large influence on risk of intramammary infections. Environmen-

tal control deals with possibilities to influence the environment of the animal in order to lower the risk of intramammary infections.

- **Therapy & Immunization**

Research on therapies, antibiotic as well as non-antibiotic, such as anti-inflammatory drug and pain control therapies, are presented in this session, including vaccination programs.

- **Control programmes**

Mastitis control elements can be integrated into mastitis control programmes. This session deals with the description of integrated mastitis control programmes for various types of dairy production systems, success factors for mastitis control programs and implementation of control programmes.

- **Mastitis control in emerging dairy countries**

Emerging dairy countries encounter mastitis problems as well as the more traditional dairy countries. However, because of the specific situations in emerging dairy countries effective mastitis control programmes may differ. Papers on specific mastitis control programmes for emerging dairy countries are presented in this session.

- **Food safety issues related to mastitis**

Food safety issues become more and more important in dairy production. This session deals with safety issues with regard to mastitis.

- **Mastitis in small ruminants**

Although bovine mastitis is the main topic of the Conference, worldwide more and more milk is produced by small ruminants (goats and sheep). In these production systems mastitis can also be a problem. This session deals with research related to mastitis in small ruminants.

The work presented in these sessions, combined with the numerous poster presentations and the satellite events provide a massive amount of information: (almost) all there is to learn about mastitis. Moreover, because of the special attention paid to practicing veterinarians, the 4th IDF International Mastitis Conference is a meeting between fundamental research, applied research, trade and practice.

For more information visit the website: www.fil-idf.org/mastitis2005

Maîtrise des infections mammaires dans les élevages agrobiologiques

Mastitis control in organic herds

L. Echevarria¹, P. Roussel², T. Cochard³, T. Brun⁴, B. Poutrel³, V. Heuchel⁵

texte français

RESUME - Les résultats des concentrations en cellules somatiques individuelles (CCSI) de 143 troupeaux d'élevages agrobiologiques adhérant au contrôle laitier de quatre régions françaises ont été analysés. Des enquêtes ont été réalisées pour apprécier la maîtrise des mammites dans 81 élevages certifiés. La faisabilité de modifications de pratiques visant à améliorer cette maîtrise a été évaluée dans neuf élevages.

A partir de l'analyse des CCSI, on a constaté une grande variabilité entre les troupeaux dans la dynamique des infections mammaires : selon les critères observés, entre un élevage sur 3 et un élevage sur 5 rencontrent des difficultés, mais la même proportion obtient des résultats très corrects. Dans un cas sur deux, on observe une dégradation pendant la période de conversion. La majorité des infections est provoquée par des germes à réservoir mammaire.

Les facteurs de conduite d'élevage apparaissant comme associés à la maîtrise des infections dans cette étude (implication des éleveurs dans le suivi du troupeau, prévention lors de la traite, ...) correspondent à des pratiques préventives ou curatives compatibles avec le cahier des charges de l'agriculture biologique. Les freins exprimés par les éleveurs vis à vis de l'adoption de pratiques de maîtrise sont plus souvent liés à l'organisation et au temps de travail qu'aux contraintes spécifiques de ce cahier des charges. Ces dernières peuvent cependant être indirectement à l'origine de freins et de difficultés de maîtrise en pesant sur la conduite globale de l'exploitation et en limitant, par exemple, le renouvellement du troupeau laitier.

English text

SUMMARY - We analysed individual somatic cell counts (ISCC) from 143 organic herds from four regions in France. Surveys were done in 81 certified organic farms with the aim to evaluate mastitis control measures. We assessed the feasibility of modifications of control measures in nine farms. The analysis of ISCC indicated that there was a great diversity in the dynamics of intra-mammary infections among herds: from 20% to 33% of the farmers had serious difficulties in controlling mastitis, but a comparable proportion managed to control intra-mammary infections. In 50% of the cases, a deterioration of the mastitis control was observed during the conversion period. Mastitis cases were mainly due to udder reservoir pathogens.

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Factors of production that seemed to be associated with mastitis control (farmers' involvement in the follow up of the health status of the herd, hygienic milking practices, ...) relate to preventive or curative practices that are compatible with organic farming requirements. What impeded dairy farmers from adopting control measures was more related to planning and work load than to the implementation of the organic farming requirements. The constraints of organic farming can, however, be the source of hindrance and difficulties to control mastitis by influencing the overall management of the farm and by limiting, for example, the renewal of the dairy herd.

Prevalence of intramammary pathogens isolated in organic herds

	Subclinical mastitis (n=473)	Clinical mastitis (n=59)
Major Pathogens		
Staph. aureus	38,4 %	40 %
Strept. dysgalactiae	2,4 %	10 %
Strept. agalactiae	0,6 %	2 %
Strept. uberis	12,4 %	24 %
Other Strept.	/	5 %
E. coli	0,2 %	10 %
Minor Pathogens		
Staph. coagulase negat	16,5 %	5 %
Corynebact. bovis	30,5 %	5 %

Continuity of Science, Communication and Wisdom

Murray W Woolford (NZ)

Woody Pankey Memorial Address
SAMM Milk Quality Conference 2004

The progress of science can be likened to the protracted international journey of the Olympic torch. Arriving at the end goal and igniting the flame of final understanding needs many runners to carry and pass on the understanding and the wisdom across time and across nations. What our new dairy science funding systems must do is to ensure that they are able to attract and nurture scientists who are capable of carrying the torch and ensuring continuity of wisdom and understanding. We need a vision of what we want to achieve and a long term strategy of how to get there. Our scientists need continuity in their careers in order to develop the vision and accumulate the wisdom and they increasingly need to be excellent communicators and listeners.

One scientist who carried the torch a long distance, and burning very brightly, was the late Dr Woodrow J. Pankey. Dr Pankey (Woody) was an internationally celebrated scientist who made a major contribution to devising and advancing science-based strategies for the control of bovine mastitis. His career epitomised the attributes outlined above. Here was a scientist who dedicated his career to the understanding and control of bovine mastitis, he was a giant among dairy scientists, and a king amongst dairy farmers, and one of the most skilled and charismatic communicators I have ever met.

My first encounter with Woody Pankey was at the old National Dairy Laboratory building at Ruakura, probably about 25 years ago, during his first NZ visit. I went there one day to talk with their micro-lab about some bug or something and Woody saw me across the lab (he was wearing one of those African hunting-safari-suite outfits that were in vogue at the time, cowboy boots and a big buckle belt), he came across and shook me vigorously by the hand saying, "*Well, if it isn't bloody Woolford*".

Wait a minute, I had never met this guy before, and I was struggling to think who the hell he was ! I don't think he knew who I was either !! But from that moment, I became aware that I was in the presence of a very unique individual. My collaborative work with Woody developed over the ensuing years and we became very solid colleagues. So, I can relate to the statement on his memorial plate at Dexcel Scott Farm today, it says; "Woody never met a stranger".

I know what that statement means, he met me as a stranger, but from that very first day, he was my friend and my colleague. Many, many other people, have since had the same experience.

Woody was born in Ruston, Louisiana, in 1944. He completed his B.S. in bacteriology in 1966 at Louisiana Tech University and followed this up in 1969 with a Master's degree, also in bacteriology. He went on to complete a PhD in 1973 entitled, "A study of Protein A from *Staphylococcus aureus* of bovine origin".

Woody was a microbiologist, "extraordinaire". He knew one hell of a lot about bugs!!

Having said that, his 1993/94 visit to New Zealand with his wife Phyllis exposed a few "knowledge gaps", in the area of field mushrooms !! You see, one of the most pleasurable times I had with Woody and Phyllis was a breakfast of field mushrooms gathered one morning, won-

dering around our country block. Woody was incredulous that in New Zealand you could go out and pick these things in the paddocks then cook them up and actually eat them without being hospitalised with food poisoning, or being arrested for consuming magic stuff!

Never to be outdone for a new angle, however, he followed up the mushroom breakfast by showing us how to create very unique "mushroom prints" by drying out large mushrooms over pieces of blotting paper in the hot-water cupboard. The spores drop out and form the most amazing geometrical patterns on the paper.

Woody had a wonderful way of communicating with farmers and farmer groups, he was highly skilled in the art. He really loved this sort of involvement and often would set up highly entertaining interactive plays to get his point across. It brought his science outcomes to fruition on farms. He really loved helping farmers sort their mastitis problems and being in there at the "gumboot level" taking samples, applying experimental treatments and commiserating with, encouraging, or giving heaps to the managers, farmers or even veterinarians, as the situation demanded!!

Many New Zealand farmers, advisers and veterinarians could recount tales of Woody's exploits in the field during his trips to New Zealand. One of them, Arney Griffiths may well remember how his bowel status was rearranged in a big way, when Woody stalled a car on railway tracks on the NZ West coast, with an advancing coal train!! Some participants of this conference may also remember the vigorous verbal sparring with guest speaker Jim Hopkins at the Milk Quality conference in Christchurch.

The mastitis research group at Ruakura/DRC enjoyed a high level of scientific interaction with Woody and Phyllis Pankey during their sabbatical visit with us in 1993-94. After their return to the US the collaboration by Email, on all kinds of topics, continued right up until Woody's last days.

Woody published over 200 reviewed scientific papers in his career and earned the absolute respect of his peers. He was one of the world's highest profile researchers of bovine mastitis. He travelled widely outside of the US and contributed enormously to the control of mastitis in many countries, including New Zealand. Many, many thousands of farmers, internationally, have benefited from his no-nonsense practical on-the-spot advice on mastitis management, advice that was always backed up with the science.

Woody held high academic office. Much of his career was spent at the Hill Farm Research Station, Homer, Louisiana where he worked with colleagues Dr Nelson Philpot and later Dr Jo Hogan. He later moved to a position at the University of Vermont with Dr John Bramley. Woody Pankey was President of the US National Mastitis Council (NMC) in 1997, and chairman of the NMC Research Committee. He was widely known among dairy scientists, commercial companies, veterinarians and in particular, dairy farmers.

In today's science environment farmers are free in their thinking about their problems and devising solutions, and in doing so they invest by way of their own time and costs. Scientists differ in that being funded and accountable, their research objectives now are closely defined and their predicted pathway towards solutions is quantified as a sequence of milestones. What is compromised in this process is the capability for reversal of thought, or changes in direction in the short term, without major legal variations in contracts. What is compromised by way of this process is the attractiveness of science as a long term career, particularly with research funding often being on a year-year basis.

What we must preserve in the NZ dairy industry is the capability for farmers and scientists to join and share the vision from both sides. To solve the big problems we need an understanding of the associated processes and mechanisms in nature. To do so, the industry must retain scientists that generate ideas and knowledge and who cannot only propagate that wisdom across time, but can also communicate outcomes to the farm situation.

Woody Pankey epitomised the ideal link between the farm and the research world. His communication skills in practical terms were legendary. On the academic side he published many papers in the best regarded journals.

I suggest to you that Woody Pankey is a role model that the New Zealand on-farm sector needs to retain in future planning, by setting up funding and nurturing structures to ensure the development and retention of such science capability.

Escherichia coli mastitis – bacterial factors and host response

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Academic dissertation 2004. University of Helsinki, Faculty of Veterinary Medicine, Department of Clinical Veterinary Sciences, Finland.

Abstract

Mastitis caused by *Escherichia coli* is common in high-producing cows with a low milk somatic cell count. The severity and outcome of *E. coli* mastitis vary between cows of the same herd and between different lactation stages in the same individual. Variation in susceptibility of cows to *E. coli* mastitis and disease severity can be caused by differences in infecting bacteria or cows' immune response. Presence of some virulence factors has previously been reported in mastitis-causing *E. coli* bacteria, with serum resistance being the most important. In early lactation, the decreased immune defence of the cow is regarded as the primary reason for increased susceptibility to *E. coli* mastitis. The aim of this thesis was to investigate bacterial and host factors affecting the severity and outcome of *E. coli* mastitis in dairy cows.

To study the bacterial factors in *E. coli* mastitis, 273 *E. coli* isolates of clinical bovine mastitis from Finland and Israel were studied by polymerase chain reaction to detect the genes for certain virulence factors. Serum resistance and capsule formation of the isolates studied were also examined as these affect the pathogenicity of the strain. The isolates possessed a variety of different virulence factors but none of them was common. The detected virulence factors, F17, S and P fimbriae, Afa8 afimbrial adhesin, cytotoxic necrotizing factor 1 and 2 (CNF1 and CNF2), aerobactin and TraT, formed 29 different combinations, including different groups of serum resistance. The association between virulence factors and the severity of the mastitis in cows affected was evaluated in Finnish material; none of the virulence factors found from the isolates was associated with the severity of clinical signs. However, presence of genes for S and P fimbriae, CNF1 and CNF2 was significantly related to persistence of mastitis. This supports the earlier findings suggesting that chronic or recurrent *E. coli* mastitis could be caused by an udder-adapted strain of *E. coli*. The results indicate that to infect the bovine udder, specific virulence factors, for example, those enhancing adhesion or invasion in the epithelia or damaging host cells are not necessary for the *E. coli* bacteria and that the virulence factors of the infecting bacterial strain probably play a minor role in the severity of clinical signs of *E. coli* mastitis.

A total of 200 of the *E. coli* isolates were also tested for antimicrobial resistance. Resistance was low; 27% of the isolates were resistant to one or more antimicrobial agents and 11% were multiresistant. All but one multiresistance pattern included resistance to tetracycline, which is often related to resistance to other antimicrobials. We also found an

association between resistance to some antimicrobials and presence of certain virulence factors. Tetracycline resistance was associated with presence of S and P fimbriae, CNF1, CNF2, aerobactin and TraT, resistance to ampicillin with aerobactin and resistance to dihydrostreptomycin with CNF2, F17 and aerobactin. These associations may cause selection of virulence factors or maintenance of antimicrobial resistance.

To study cow factors and host response we used an experimental *E. coli* endotoxin model in which nine cows were challenged twice, in early lactation (EL) and in late lactation (LL). Cows showed a significantly more severe response in the EL than in the LL period. The difference was seen in systemic signs, whereas local signs were similar for both periods. The concentration of tumour necrosis alpha (TNF α) in milk increased after the endotoxin challenge, reaching higher levels in EL cows. No TNF α was detected in blood. Although TNF α might not be directly responsible for the systemic signs in endotoxin mastitis, it probably has a critical role in initiating host response by inducing the local production of other cytokines, which in turn mediate the systemic effects of the host response. TNF α also seems to induce production of serum amyloid A (SAA) in milk, as milk SAA concentration was higher in EL than in LL, and the concentrations of TNF α and SAA were closely related at the cow level. In blood, the SAA increased later, after endotoxin challenge, with the average concentration being higher in LL, indicating differences in production and regulation of local and systemic SAA.

After induction of endotoxin mastitis, the number of polymorphonuclear leucocytes (PMN) in milk and blood increased faster, reaching higher levels in the EL than in the LL period. However, the function of blood neutrophils after endotoxin challenge, measured as chemiluminescence (CL), increased in LL but decreased in EL. This impaired function of blood PMN in EL could be caused by subclinical ketosis of the cow, reflected in the increased serum free fatty acid concentrations. The CL of milk PMN increased in both EL and LL, being more pronounced in EL and appearing simultaneously with the decreased CL of PMN in blood. This may have resulted from an influx of the most active PMN from the circulation to the milk and from the stimulation of the milk PMN by such locally produced cytokines as TNF α . The function of PMN may though be more critical for host defence and for susceptibility of cows to *E. coli* mastitis than their actual number in blood and milk.

The results of this thesis support the findings of previous studies showing that the increased susceptibility of the cow to *E. coli* mastitis and the more severe course of disease in early lactation depends more on host factors than on the characteristics of the infecting bacterial strain. The virulence and antimicrobial resistance of mastitis-causing *E. coli* reflect the situation of bacteria found in the environment or in the intestines or faeces of the cow. To prevent *E. coli* mastitis, efforts should be focused on improving the environment of the cows and herd management.

Diagnostic Potential of the California Mastitis Test to Detect Subclinical Mastitis

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Basically, the determination of the udder quarters health status requires measurements to be made of the presence or absence of microbiological pathogens in the milk and of inflammation-related changes. The somatic cell count (SCC) has been chosen as the best indicator for the inflammatory response [1]. The methods used depend on the objectives of the work: While surveys and control programmes may also be carried out by applying indirect methods, experimental and observational studies require measurements of the SCC in foremilk samples, preferably by using electronic methods [2]. But, with the exception of the *Direct Cell Counter (DCC, DeLaval Co., Sweden)*, electronic counting systems for somatic cells are laboratory-based and therefore time-consuming. Furthermore, compared with indirect tests all direct cell counting methods are costly. As a consequence the California Mastitis Test (CMT), as a cow-side test, may enable the bovine practitioner to detect inflamed udder quarters promptly for immediate therapeutic or management-related action. Since the prevention of mastitis during the dry period has become more important by the application of external or internal teat-sealants, a paradigm shift has taken place: the use of diagnostic procedures, methods or tests (for example, CMT) intends to detect "healthy" cows [3, 4], that is, cows with four normally secreting udder quarters. Concerning the CMT *Schalm and Noorlander* [5] pointed out, already in 1957: "In normal foremilk, that is milk negative to the test, the mean total count did not exceed 100 000 cells per milliliter of milk¹. " In order to characterize the physiological pattern of milk secretions, numerous authors confirmed this observation from different perspectives, for example, concentration of cells [6], milk composition [7, 8], milk processability [9] and yield [10]. Latest information on the basis of 178 374 cow composite milk samples (CCM) clearly demonstrated that even an increase from less than 50 000 cells up to 100 000 cells/ml CCM resulted in a milk loss of three percent [11].

¹During discussions in the Standing Committee Animal Health of the IDF, the following considerations were made. It is internationally accepted that a cow somatic cell count under 100,000 cells/ml can be regarded as healthy. Due to deviation in the measurement of somatic cell count, a measured somatic cell count higher than 200.000 cells/ml can be regarded as abnormal. The area between 100.000 and 200.000 cells can be regarded as „gray“.

The principle of the CMT consists in mixing the test reagent with an equal quantity of milk and reading the reaction (expressed as the intensity and amount of gel produced) depending on the number of somatic cells (or their DNA) in visual scores as 0, Trace (T), 1, 2 or 3. Considering several publications [12, 13, 14], the CMT-scores seem not to reflect a satisfactory differentiation between inflamed and uninflamed udder quarters based on a SCC threshold of 100 000 cells/ml. In this case, the CMT would be an inappropriate diagnostic test for the determination of the udder quarter's health. Therefore, the present study deals with the evaluation of the diagnostic potential of the California Mastitis Test performed under defined laboratory conditions.

Present Study

Material and Methods

A total of 107 German Holstein cows at different lactation stages and lactation numbers were examined. The total sampling included 1426 quarter foremilk (QFM) samples of clinically inconspicuous quarters and 25 QFM samples of clinically affected ones. Sampling was always performed at morning milking time. After discarding the first stripped milk jets, all teats per cow were cleaned with dry paper tissues and the teat apices were disinfected using ethyl alcohol (70 Vol. %). After the occurrence of complete milk ejection, approx. 10 ml foremilk per lactating quarter were milked separately into a glass tube. Within two hours, samples were brought to the institute and stored in the cooling chamber (+4°C). The somatic cells were counted applying the fluorescence-optical method of the *Fossomatic 360* (Foss Electrics Co., Denmark). Microbiological examinations were performed considering the guidelines for isolating and identifying mastitis pathogens of the German Veterinary Medicine Association [15]. According to IDF, the udder health status was categorized as "normal secretion", "latent infection", "unspecific mastitis" or "mastitis" [16] using a SCC threshold of 100 000 cells/ml QFM. Each diagnosis at quarter level was based on the cytological and microbiological findings of one sampling day.

Under laboratory conditions, the mixing ratio between CMT reagent and test milk was precisely 1:1. Using pipettors (*Eppendorf Co., Germany*), volumes of 2 ml for both CMT reagent and test milk were pipetted into the cups of the testing paddle (definite volume of mixture per cup: 4 ml). Milk and reagent were mixed by gently swaying the paddle. The testing paddle was tilted to decant one half of the mixture, so to detect minor changes more easily. Readings were done under good lighting conditions within 20 seconds by tilting the paddle. To identify inflammation-related changes by the CMT, only two scores are needed: 1. Negative = no precipitates, 2. Positive = (even slightest) precipitates.

In order to determine variables influencing the California Mastitis Test, additional studies were performed:

- 1st Two commercially available CMT reagents were compared based on identical QFM samples (n = 116).
- 2nd In order to assess the comparability of CMT results under laboratory and field conditions, two studies were carried out: First, samples of two different milk fractions per quarter were taken (QFM samples and the subsequent approx. 90 ml secretion milked by hand into a glass flask [QMH], n = 100 quarters) to evaluate a fraction-related influence on CMT results within the first 100 ml milk per quarter. Based on the results of the first study, a total of 407 quarters were tested twice. Initially, the CMT was applied in the milking parlour. Subsequently, QFM samples of the identical quarters were taken and examined afterwards under laboratory conditions.
- 3rd Under practical conditions it may be advantageous to sample milk probes for later examination in order to save additional work during the regular milking time. For this purpose, preservation of the somatic cells is required. Therefore, CMT results of milk preserved with bronopol and sodium acid, respectively, were compared with those of

native milk (identical fractions of $n = 100$ QMH samples), considering a 24-hours storage time.

4th The examiner's individual influence on CMT readings was evaluated. Three examiners, all adept in applying the CMT, tested the identical QMH samples ($n = 100$) blindly.

5th Manually dosed, the required mixing ratio of equal CMT reagent and test milk volumes is not ensured. Therefore, the influence of CMT reagent underdoses and overdoses, respectively, on CMT results was assessed. Using identical fractions of 100 QMH samples, four different mixing ratios between CMT reagent and test milk were compared with each other (applied mixing ratios: 1:4, 1:2, 1:1, 3:2).

All measurements were carried out blindly. Data recording and processing were performed using *Microsoft Excel* (Microsoft Co., USA). Data was evaluated statistically applying the scientific software *SAS 8e* (SAS Institute Inc., USA) and *BiAS 7.07* (University Frankfurt a. M., Germany). The probability of error α is 1% ($p \leq 0.01$). The specific statistical procedures will be mentioned in connection with the presented probability values.

Results

The evaluation of influencing variables of the CMT mentioned above can be summarized as follows:

1st study: Slight differences between two commercially available CMT reagents were not statistically significant ($n = 116$ QFM: *Fisher's exact test*, $p = 0.232$). Therefore, further studies were performed using the identical CMT reagent.

2nd study: Differences between QFM samples and QMH samples within the first 100 ml milk per quarter were not statistically different, neither for CMT reactions nor for cell counts ($n = 100$ quarters: SCC: *Student's t-test* for two independent samples, $p = 0.458$; CMT: *Fisher's exact test*, $p = 0.877$). Therefore, it is feasible to compare the CMT procedures under laboratory and field conditions as described above. The comparison of both CMT procedures showed no statistically significant differences ($n = 407$ quarters: *Fisher's exact test*, $p = 0.145$).

3rd study: Concerning the evaluation of CMT results under consideration of somatic cells preserving reagents and storage time, respectively, the application of the *non-parametric analysis of variance by Friedman* led to statistically significant differences ($n = 100$ QMH: $p < 0.001$). Within a storage time of up to three hours, no significant influences on CMT results were found neither between both preserving reagents nor between preserved and native milk. Yet, significantly different CMT results occurred after a storage time of 24 hours (bronopol vs. sodium acid: *comparisons by Schaich and Hamerle*, $p < 0.001$). The storage time itself influenced the CMT results of native milk and samples preserved with bronopol (0 h vs. 24 h: *comparisons by Schaich and Hamerle*, $p < 0.001$). In contrast, CMT results of QMH preserved with sodium acid remained comparable (0 h vs. 24 h: *comparisons by Schaich and Hamerle*, $p < 0.451$).

4th study: Examiner's individual influences on CMT results were statistically significant ($n = 100$ QMH: *nonparametric analysis of variance by Friedman*, $p < 0.001$).

5th study: The mixing ratio between CMT reagent and test milk influenced the CMT readings significantly ($n = 100$ QMH: *non-parametric analysis of variance by Friedman*, $p < 0.001$). Figure 1 shows the results.

The evaluation of the diagnostic potential of the California Mastitis Test included the findings of 1,426 QFM samples of clinically inconspicuous quarters and 25 QFM samples of clinically affected ones. In order to obtain more precise results, the commonly used CMT scores were augmented as shown in Table 1. Along with these modified scores, the corresponding cell counts of 1,426 clinically inconspicuous QFM samples are detailed (Table 1). The occurrence of even the slightest precipitates leads to a positive result (Cut-off: ≥ 1). To verify this decision, the CMT results were contrasted with the udder health categories (Table 2). As shown there, 95% of all subclinically inflamed quarters (538 out of 565 cases of mastitis and unspecific mastitis) were detected using the chosen cut-off level. Therefore,

including even the slightest smears in positive CMT results seems to be suitable. The most frequent CMT failures were seen for "latent infections".

Results of the CMT were compared with corresponding SCC values based on the fourfold table. Cut-off levels for CMT and SCC were fixed on score 1 and 100 000 cells/ml, respectively. Table 3 shows the fourfold table. The corresponding basic statistics can be specified as follows: prevalence (39.6%), sensitivity (95.2%), specificity (68.4%), diagnostic accuracy (79.0%), Youden index (0.636).

Under field conditions, the very low number of false negative CMT results (27 out of 565 diseased cases) is highly important for CMT practicability because these cases represent undetected diseased quarters. Considering SCC ranges, the percentage of false negatives is extreme low, even in a SCC range between 100 000 and 200 000 cells/ml milk (9.9%).

Implications

There is still a serious need for suitable, quick and preferably inexpensive diagnostic tests to screen the herd at quarter level and at shorter intervals in order to assess the udder health status promptly for immediate therapeutic or management-related action. As demonstrated, the California Mastitis Test allows a reliable detection of sub-clinically inflamed udder quarters, even for sub-clinical mastitis cases shortly above the physiological threshold of 100 000 somatic cells/ml. In contrast to these results, commonly stated cell count levels corresponding with negative CMT results range up to 200 000 cells/ml, and up to 500 000 cells/ml for trace CMT results respectively [13, 14]. To obtain standardized CMT results, the following aspects should be regarded strictly:

- 1st The first milk jets have to be discharged (for example, strip cup).
- 2nd Only foremilk after occurrence of complete milk ejection should be used.
- 3rd Add an equal amount of CMT reagent to the test milk in the cup. Avoid underdoses!
- 4th Mix milk with the reagent by gently swaying the paddle.
- 5th Detecting minor changes becomes difficult, if more than a volume of 2 ml of reagent-milk solution is in the cup; if so, decant the supernatant carefully until all four marks inside the cups reappear.
- 6th Readings should be done under good lighting conditions and within 20 seconds by tilting the paddle.
- 7th Operators performing the CMT should have sufficient experience.
- 8th It is recommended to consider only two CMT scores:
 1. NEGATIVE = no precipitates and 2. POSITIVE = precipitates (even the slightest smears).

Under practical consideration, more detailed CMT scores will not result in additionally needed information. In contrast, such scores imply the risk of herd management failures if treatment procedures only include the highest scored cows.

In order to detect sub-clinical mastitis, the best diagnostic test would offer a sensitivity as well as a specificity of 100%. A lower specificity implies false-positive quarters, that is, "healthy" quarters erroneously regarded as inflamed. Such quarters may cause economic losses due to unnecessary treatment-related costs. On the other hand, a lower sensitivity describes false-negative quarters. These sub-clinically inflamed quarters remain undetected. At least concerning contagious udder pathogens, such quarters extensively increase the risk of new infections within a herd. With regard to all infection-related implications, a high sensitivity is a precondition for a successful herd health management. With a sensitivity of 95%, the California Mastitis Test roughly fulfils this requirement.

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Tables and Figures

Table 1: Applied CMT-scores and corresponding cell counts of 1426 clinically inconspicuous quarters (QFM samples)

Results	California Mastitis Test		Corresponding cell counts					
	Readings	Score	Number of samples			log ₁₀ SCC/ml		SCC/ml
			TOTAL (100%)	≤ 100,000 (%)	> 100,000 (%)	X _a	± sd	X _g
Negative	No smears	– (0)	222	218 (98.2)	4 (1.8)	4.084	0.417	12,134
Cut off	Questionable	± (0,5)	394	371 (94.1)	23 (5.8)	4.264	0.443	18,365
	Slight smears	+ (1)	301	207 (68.8)	94 (31.2)	4.761	0.462	57,677
Positive	Moderate smears	++ (2)	245	61 (24.9)	184 (75.1)	5.234	0.484	171,396
	Strong smears	+++ (3)	171	4 (2.3)	167 (97.7)	5.600	0.327	398,107
	Commencing gelation	++++ (4)	63	0 (0.0)	63 (100.0)	5.982	0.335	959,401
	Gelatin	+++++ (5)	30	0 (0.0)	30 (100.0)	6.485	0.406	3,054,921
	Visibly abnormal milk	Flakes (6)	25	2 (8.0)	23 (92.0)	visible changes		

Table 2: Comparison of CMT results depending on udder health categories (n = 1426 clinically inconspicuous quarters, QFM samples)

Somatic cell count (SCC) [cells/ml milk]		Udder pathogens	
		not detected	detected
≤ 100,000	n diagnoses	Normal secretion 642	Latent infection 219
	log ₁₀ X _a ± sd	4.189 ± 0.397	4.547 ± 0.374
	X _g	15,453	35,237
	CMT score: ≥ 1	24.9%	51.1%
	CMT score: < 1	75.1%	48.9%
> 100,000	n diagnoses	Unspecific mastitis 192	Mastitis 373
	log ₁₀ X _a ± sd	5.582 ± 0.496	5.562 ± 0.390
	X _g	381,944	364,754
	CMT score: ≥ 1	93.2%	96.3%
	CMT score: < 1	6.8%	3.7%

Table 3: Comparison of CMT results and SCC values by means of the fourfold table and considering SCC ranges (n = 1426 clinically inconspicuous quarters, QFM samples)

SCC [cells/ml milk]	CMT		n Quarters
	positive (≥ 1)	negative (< 1)	
$\leq 100,000$	False positive 31.6% n = 272	True negative 68.4% n = 589	"Healthy" 861
$> 100,000$	True positive 95.2% n = 538	False negative 4.8% n = 27	Diseased 565
Of those			
up to 200,000	90.1% n = 164	9.9% n = 18	182
up to 300,000	96.2% n = 100	3.8% n = 4	104
up to 400,000	98.2% n = 54	1.8% n = 1	55
$> 400,000$	98.2% n = 220	1.8% n = 4	224

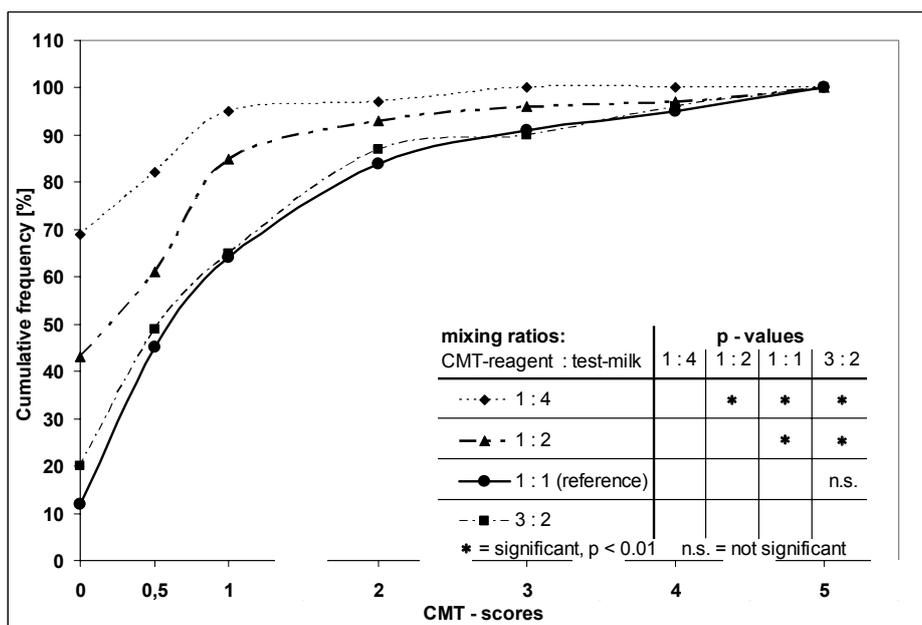


Figure 1: CMT results depending on different mixing ratios (n = 100 QMH samples; multiple comparisons by Schaich and Hamerle, p-values)

Role of lactoferrin in treatment of bovine mastitis

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Abstract

The non-specific, multifunctional glycoprotein lactoferrin (Lf) is present in milk and external body secretions. It is released by the secondary granules of neutrophils and epithelial cells in high concentrations in response to inflammatory stimuli. Lf has a broad-spectrum antimicrobial activity, especially against coliform bacteria, such as *Escherichia coli*, which cause severe mastitis in dairy cows. Since treatment of severe coliform mastitis using antimicrobial agents is problematic, Lf as a natural protein may offer an alternative for treating this disease.

The aim of this work was to evaluate the usefulness of exogenic Lf in mastitis treatment. First, normal concentrations of Lf and citrate in the early dry cow secretion of healthy cows were studied. Second, the antimicrobial efficacy of Lf against udder pathogens, particularly *E. coli*, was tested *in vitro*. Third, the disposition kinetics of Lf infused into the udder quarters of dairy cows was investigated. Finally, the antibacterial effect of Lf was studied in an experimental *E. coli* mastitis model.

To determine the normal concentrations of Lf and citrate, milk and dry udder secretion samples were collected on the last day of lactation before drying-off, and then 2 and 6 days later. The mean Lf concentration in the milk increased from 5.29 mg/ml on the last day of drying-off to 8.09 and 11.26 mg/ml 2 and 6 days later, respectively. Citrate concentration decreased from 1.85 mg/ml to 1.54 and 1.09 mg/ml, respectively. Concentrations varied greatly between cows and even between udder quarters at each point in time. Median molar ratio (citrate to native Lf) decreased gradually from 153 on the last day of lactation to 44 on day 6 after drying-off.

The antibacterial effect of Lf was tested *in vitro* against *E. coli*, *Staphylococcus aureus*, and coagulase-negative staphylococci (CNS) as well as on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* originally isolated from bovine mastitis. Concentrations of Lf used were 0.67, 1.67 and 2.67 mg/ml. The best inhibitory activity of Lf was seen against *E. coli* and *P. aeruginosa*. The inhibitory effect of Lf for *E. coli* was concentration-dependent and variation between the five isolates of *E. coli* was small. None of the isolates was totally resistant to Lf. The growth of two isolates of *P. aeruginosa* was clearly inhibited in Iso-Sensitest Broth, in contrast to two *K. pneumoniae* isolates which were virtually unaffected. The isolates of CNS and *S. aureus* showed more variation in susceptibility to Lf than *E. coli*. Three isolates of *S. aureus* were more susceptible to Lf than the two other isolates at 0.67 mg/ml of Lf. The growth of four CNS isolates was somewhat inhibited by Lf but one isolate was totally resistant.

The disposition kinetics of Lf after intramammary administration was studied in lactating dairy cows. A 1 gram dose of Lf produced elevated Lf concentrations in milk for several hours. The mean elimination half-life of total Lf was 2.2 h and the mean maximum concentration of 6.3 mg/ml was reached between 1 and 4 h post-infusion. The average Lf concentration in milk of six cows before administration of Lf was 0.4 mg/ml. After 8 h of administration the average Lf concentration decreased to 0.8 mg/ml. Lf caused some local tissue irritation in the udder quarters but general signs, such as fever and anorexia, were not observed. The udder quarters of primiparous cows seemed to react faster than those of multiparous cows. The irritation reactions decreased more rapidly in older cows than in primiparous cows.

In an experimental *E. coli* mastitis model the clinical response to the challenge varied markedly among individual cows. In general, systemic signs disappeared within 2-3 days, and local signs within one week. Differences in systemic and local clinical signs between Lf- and enrofloxacin-treated cows were not significant. Bacterial counts in milk decreased faster in enrofloxacin-treated than Lf-treated cows. The difference almost reaching significance. Bacteria were eliminated from the challenged quarters of the enrofloxacin-treated cows within 3.8 days and Lf-treated cows within 5.8 days, on average. Differences in somatic cell counts between treatments were not significant. However, NAGase activity remained high for a longer time in milk of cows treated with Lf than with enrofloxacin. Daily and quarter milk yield profiles over the experimental period were similar in both treatment groups.

Monitoring Bovine Mastitis in Finland – changes in prevalence from 1988 to 2001

A. Pitkälä¹, M. Haveri², S. Pyörälä², V. Myllys¹, and T. Honkanen-Buzalski¹

Despite of implementation of control strategies, bovine mastitis has remained the major challenge to the dairy industry worldwide. In Finland, approximately 30 % of the veterinary visits on the farms are made for mastitis. In many countries, the prevalence of contagious bacteria, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, has reduced, and coagulase-negative staphylococci and *Corynebacterium bovis*, traditionally considered as minor mastitis pathogens, have become more common.

Bulk milk somatic cell count (BMSCC) has been regularly recorded in Finland since 1980s, and nationwide monitoring to estimate prevalence of bovine mastitis and distribution of mastitis pathogens involved has been conducted since 1988. During recent years the BMSCC has remained at a stable level, the geometric mean being approximately 132 000 cells/ml. Bovine mastitis prevalence surveys were made 1988, 1995 and 2001. The farms included each year were randomly selected from a database covering all Finnish dairy farms. Quarter milk samples collected by the dairy advisors were submitted for somatic cell counting (1995 and 2001) or CMT measurement (1988), bacteriological examination and testing for antimicrobial susceptibility. If the milk somatic cell count of a cow or of a quarter exceeded 300 000/ml³, the cow was defined as having mastitis. A milk sample was considered as bacteriological positive when growth of ≥ 500 cfu/mL was detected from a sample.

The herd prevalence of bovine mastitis has decreased continuously from 48% in 1988 to 31% in 2001. Comparing the survey results 1988, 1995 and 2001, the proportions of the main mastitis pathogens have changed also in Finland through the years (Table 1). Coagulase-negative staphylococci has become the most common bacterial group, comprising almost half of the pathogens isolated in 2001. The relative number of *Staphylococcus aureus* isolations has decreased from 1988 study, but its prevalence at cow or quarter level has been stable since 1995. The proportion of bacteriological positive quarters has increased. In 1988, 83% of the samples were considered bacteriologically negative; the corresponding figure was 79% in 1995 and 62 % in 2001. This mainly resulted from increased prevalence of *Corynebacterium bovis*.

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3 This definition deviates from IDF definitions, in which a mastitis case is defined as a cow with a cow somatic cell count higher than 500,000 cells confirmed with the isolation of a mastitis pathogen in the milk of one or more udder quarters. When these events are combined with visible changes in milk and/or udder, it is a clinical mastitis case, otherwise a subclinical mastitis case.

Source:

Griffin, T.K., Morant, S.V. and Dodd, F.H. 1987. Diagnosing infectious subclinical mastitis in surveys or large scale experiments. The analysis and interpretation of the results of an international trial organised by the IDF Mastitis Expert Group (A2). Bulletin of the International Dairy Federation, 211: 9-24.

The trends in susceptibility were similar to published data from other countries. From 1988 to 2001, the proportion of strains resistant to at least one antimicrobial drug increased both among *S. aureus* and among CNS, most of the change being due to higher number of penicillin-resistant isolates. Penicillin resistance among the staphylococci in Finland in 2001 was 52.1% for *Staphylococcus aureus* and 32.0% for coagulase-negative staphylococci. In other Nordic countries more than 90 % (Norway) or more than 80 % (Sweden) of the isolates are susceptible to penicillin and the situation has remained stable for decades. The possible reasons can be speculated. In Norway and Sweden mainly penicillin G has been used and only a few intramammary preparations have been available. In Finland, in 1988 a total of 29 intramammary preparations with 19 different antimicrobials were on the market. Streptococci isolated from mastitis were very susceptible to β -lactam antibiotics, as was also found in the previous survey in 1995. According to *in vitro* antimicrobial susceptibility testing, the enterococci demonstrated the highest level of resistance.

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PITKÄLÄ, A., HAVERI, M., PYÖRÄLÄ, S., MYLLYS V. & HONKANEN-BUZALSKI, T. 2004. Bovine Mastitis in Finland 2001 – Prevalence, Distribution of Bacteria, and Antimicrobial Resistance. *J. Dairy Sci.* 87: 2433-2441.

Table 1. Proportions of different pathogens isolated during the surveys in 1988, 1995 and 2001.

	1988 n=2803	1995 n=2172	2001 n=4237
CNS ¹	39.78	53.50	49.63
Staphylococcus aureus	31.00	16.67	10.17
Streptococcus agalactiae	4.71	0.55	0.07
Streptococcus dysgalactiae	4.78	0.37	0.14
Streptococcus uberis	7.14	3.41	1.94
Other streptococci	5.28	6.86	0.21
Enterococcus spp. ²			1.20
Aerococcus viridans ²			0.71
Lactococcus spp. ²			0.73
Corynebacterium bovis	4.99	16.62	34.41
Coliforms	1.57	1.38	0.42
Other bacteria	0.75	0.64	0.37
Total	100.00	100.00	100.00

¹ = Coagulase-negative staphylococci

² In 1988 and 1995, included as other streptococci

10 years of inter-comparisons on counting of somatic cells in raw milk

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Somatic cell count (SCC) is an important parameter for the hygienic quality of raw milk. It is also used in payment systems for ex-farm milk. Routinely SCC in raw milk is determined by fluoro-optoelectronic methods based on flow cytometry or rotating disc. Inter-laboratory trials are an important tool to check the proficiency of laboratories. These inter-comparisons intend to ensure identical results when identical samples are analysed by different laboratories.

The German reference laboratory for counting of somatic cells in raw milk at the Federal Research Centre for Nutrition and Food (former Federal Dairy Research Centre) in Kiel has carried out yearly IDF inter-comparisons for more than 25 years in addition to national ring trials. In these trials 9 to 10 milk samples with different levels of somatic cell count from low level to approximately 1 000 000/ml are provided. Based on splitting each sample into 4 sub-samples, coding at random and 4-fold counting of each sub-sample repeatability (r) and reproducibility (R) are determined.

As examples for the results the outcome of the international ring trials no. 24 and 33 regarding maximum repeatability and maximum bias for individual laboratories are presented in Figure1.

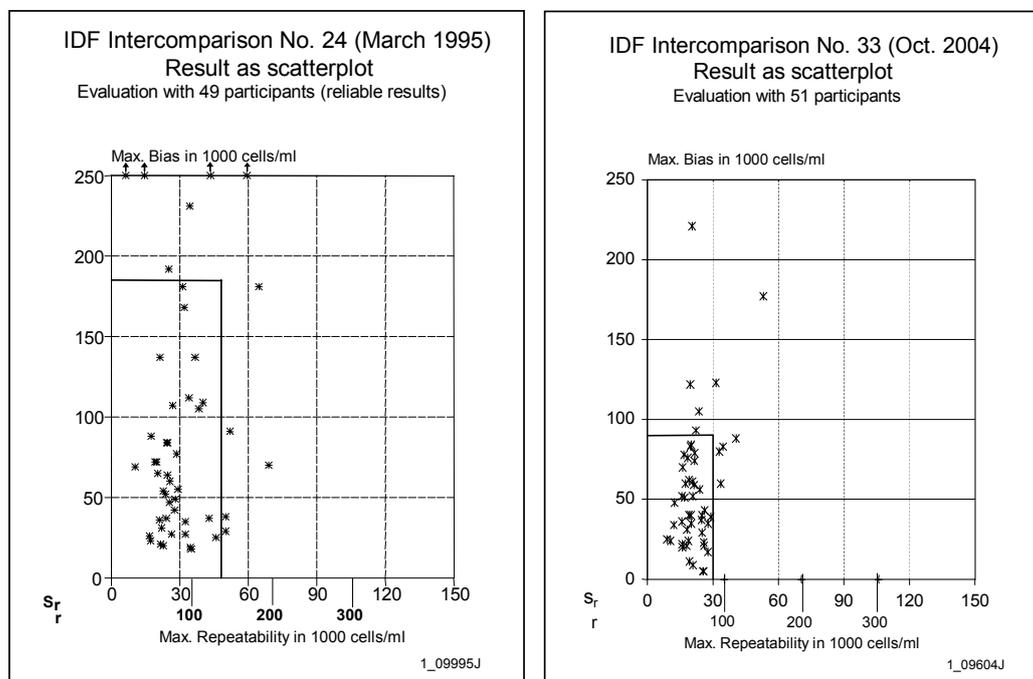


Figure 1: Maximum repeatability and bias for individual laboratories participating in IDF inter-comparisons on counting of somatic cells in raw milk in 1995 and 2004 (marked area including 85 % of participants); s_r = standard deviation reporting repeatability, bias = systematic error (difference from true value)

Although the number of laboratories showing extremely high maximal bias has decreased between 1995 and 2004, the results of 2004 are not quite satisfactory. The progress in proficiency of laboratories between 1994 and 2004 is summarized for the national and international ring trials in Table 1.

Table 1: Progress in proficiency of laboratories in counting of somatic cells expressed by 85%-level (distribution of participants)

year	national (Germany)			international (IDF)		
	no. of participants	s_r	bias	no. of participants	s_r	bias
1994	53	40	160	42	103	300
1995	58	42	67	49	48	186
1996	55	61	121	61	43	127
1997	52	35	59	67	37	118
1998	70	28	72	60	38	107
1999	64	32	62	65	30	98
2000	60	24	47	65	31	76
2001	56	26	60	56	35	96
2002	55	26	58	48	29	78
2003	57	28	46	45	40	122
2004	58	26	57	51	29	85

s_r = standard deviation reporting repeatability (in 1000 cells/ml); bias = systematic error (difference from true value, in 1000 cells/ml), for each participant the worst case of 9 (or 10, respectively) milk samples was taken;

National trial 1996: leap at 82% portion of participants;

International trial 2001: several labs reporting difficulties were excluded

In general, repeatability is satisfactory at national level but it can be improved at international level. The international ring trials also showed systematic errors in a significant number of laboratories throughout the whole time period. In contrast, bias has been continuously reduced at national level in Germany. This has been achieved by routine use of reference material and by improving lab-proficiency in general. At international level further harmonization is needed to avoid trade restrictions. Common requirements for quality of the (secondary) reference materials used for calibration and control of instruments would probably facilitate harmonization. Furthermore, from such harmonization, the use of SCC in determining mastitis on the basis of quarter milk samples would also benefit.

Microscopic Counting of Somatic Cells in Milk

Ernst-Heinrich Ubben¹, Karin Knappstein¹, Bertrand Lombard²

Workshop of the European National Reference Laboratories "Milk and Milk Products"
at the Federal Research Centre for Nutrition and Food, Kiel, Germany
September 9-10, 2004

The workshop was dedicated to the microscopic method of enumeration of somatic cells in milk as the reference method for official controls, described in part 1 of the International Standard IDF 148/ISO 13366. In Europe, somatic cell count (SCC) is a criterion for raw cow milk which is currently set by the Milk Hygiene Directive 92/46/EEC, and maintained in Regulation 853/2004, the new hygiene legislation which will take over the Directive 92/46.

The background to the workshop was a European inter-laboratory trial on the counting of somatic cells in liquid raw milk, organized in November 2002 by the European Community Reference Laboratory "Milk and Milk products" (CRL Milk, located in AFSSA-LERQAP, Maisons-Alfort, France) for the National Reference Laboratories (NRLs). This trial identified difficulties with the practical implementation of the reference method. The workshop was co-organized by Bertrand Lombard from the CRL Milk and by Ernst-Heinrich Ubben from the German reference laboratory for counting of somatic cells in raw milk. Delegates from the NRLs from Austria, Belgium, Bulgaria, Cyprus, Estonia, Finland, France, Germany, Greece, Ireland, Italy, Lithuania, Malta, the Netherlands, Poland, Portugal, Slovakia, Spain and Switzerland, as well as from EU DG SANCO and on behalf of IDF attended the workshop.

Objectives of the workshop were:

- to reach a common way and level of competence amongst the NRLs for the implementation of the Standard microscopic method;
- to discuss critical points of the method and to envisage ways to improve them;
- to establish a link with IDF/ISO and to contribute to the revision of the Standard IDF 148/ISO 13366-1;
- to open a broader perspective on the use of SCC.

Practical training during the workshop was accomplished by lectures on

- Relevance of control of somatic cells in milk with regard to udder health and product quality (Karin Knappstein, DE);
- Cell differentiation in bovine milk (Anke Schroeder, DE);
- ISO Standard 13366, its renewal and prospect of the new version (Silvia Orlandini, IT for IDF);
- Quality management with focus on ring trials (Ernst-Heinrich Ubben, DE).

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The laboratory procedure was discussed according to the experiences of the delegates from practical training. Proposals on description of the method regarding apparatus to be used, preparation of the smear, drying of the film and cell counting were made to be included in the revision of Standard IDF 148/ISO 13366-1, which is under development by IDF/ISO Joint Action Team "Automated Methods".

For the European NRLs a new phase of proficiency testing on microscopic counting of SCC is planned for 2006. Several NRLs reported that instead of the microscopic reference method different kinds of reference materials are used for routine calibration of instruments. Accordingly a follow up workshop is envisaged on the use of alternative methods for the control of raw milk. Special emphasis will be laid on calibration of alternative methods against a reference method or reference material, respectively, and on the homogeneity of reference materials. This workshop will be dedicated not only to SCC but also to total bacterial count because the routine methodology for determination of both parameters is similar.

Reference

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Influence of storage conditions of antibiotics on excretion time in milk

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Different causes for positive inhibitor tests of bulk tank milk have been reported: delivery of milk from treated cows during the withholding period, failures during milking, improper cleaning of equipment as well as treatment errors and extra label use of antibiotics (Schälibaum, 1990; Fabre et al., 1995). In some cases prolonged excretion in milk of individual cows is suspected. Limited information is available on the influence of storage conditions on the excretion time in milk although for veterinary drugs usually storage temperatures are recommended by the manufacturers.

Our own investigations were performed on the excretion of cefquinome in milk in relation to storage conditions of Cobactan® LC. According to the manufacturer (Intervet Int., Unterschleissheim, DE) the storage temperature should not exceed 20 °C. Healthy cows were treated with properly stored injectors in all 4 quarters during three subsequent milking times (one injector per udder quarter). In a second experiment 6 cows were treated with heat-treated injectors. The drugs were stored on the radiator over night. The concentration of residues in milk were determined by LC methods (Suhren and Knappstein, 2003) during the experimental period including anamnesis, treatment period, withholding period and additional milking times. The results are shown in figure 1.

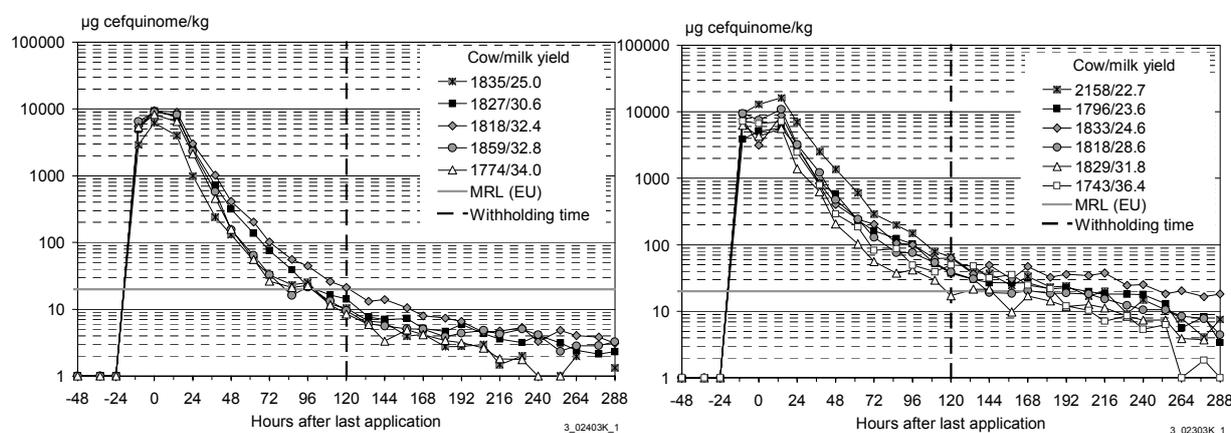


Figure 1: Excretion of cefquinome residues in milk after treatment with Cobactan LC; left: properly stored injectors, right: heat treated injectors

After treatment with the mishandled injectors cefquinome excretion in milk was markedly prolonged. Concentrations exceeding the Maximum Residue Limit (MRL) in milk of 20 µg/kg (EU) were detected in milk of all 6 cows up to 120 hours after the end of the withholding period.

According to an analysis of the manufacturer the content of the heat-treated injectors showed macroscopic changes in colour and homogeneity as well as an increased viscosity, although the cefquinome content complied with shelf-life specifications. Probably the changes in viscosity lead to retarded release of cefquinome and thus to an extended withdrawal time.

These results indicate that proper storage of antibiotic drugs is highly important to ensure that withholding periods for milk are not exceeded.

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Five year project to reduce mastitis in The Netherlands

Theo Lam (NL)

Animal Health Service, Deventer, the Netherlands

As in other countries, udder health is a factor of major importance for Dutch dairy farmers. Over the last few years it was found that bulk milk SCC increased, with high peaks in summer, and that clinical mastitis remained a serious problem in many herds. Moreover there is a growing concern for animal welfare, public health and milk quality. Currently the percentage of clinical mastitis cows in the Netherlands is estimated to be at least 25%. Dutch farmers are not satisfied with that, and want to be at the forefront in respect of udder health and milk quality. To achieve this goal the Committee on Animal Health and Quality of Cattle has given the Animal Health Service the task to reduce this percentage by 10% to 15% within five years.

The project consists of three parts. The first part is implementation, where we want to get the existing knowledge across. We want farmers to do what they know they should do. The second part of the project is research orientated. For this a research committee has been appointed consisting of a variety of experts on all aspects of mastitis. Finally, as third part, the Dutch Udder Health Centre has been established to coordinate all activities. This Centre will be located at the Animal Health Service in Deventer.

An orientating study among 400 dairy farmers demonstrated that they consider their local veterinary practitioner as the first person to consult whenever they experience mastitis problems. Therefore the private practitioner was chosen as the axis of the implementation part of the project. During 2005 a pilot exercise will be done in 10 veterinary practices with approximately 1500 dairy farms. The goal of this year's work is to find out whether or not the practitioners are able to motivate farmers enough to improve udder health in all herds in their practice. The Udder Health Centre will provide the practitioners and farmers with all kinds of instruments and knowledge. Dry cow therapies, prevention strategies, milking hygiene and milking routines will be evaluated as to their contribution to the udder health improvement. Progress will vary from herd to herd and from practice to practice. To determine the udder health status at the level of the herd as well as at the level of the veterinary practice, data will be gathered on clinical mastitis, individual cow SCC and bulk milk SCC.

During 2004 the research committee identified fields of research needed to improve udder health on dairy farms. The main focus is on applied research. How can we help the farmer to manage his mastitis problem? Subjects studied will be in the field of diagnostics (optimization of classical microbiology, modern techniques), economics (diagnostics, treatments, projects like this one), factors influencing resistance of cows (at cow level, at herd level), and some other fields. An important field of research will also be Communication. How can we motivate farmers to do what they know they should do? How can we motivate practitioners to motivate their clients? Is it only economic pressure that drives them or are there other factors?

Altogether this project will be one of the largest ever executed in this field in the Netherlands. It has a quantified goal that makes it an enormous challenge. The forthcoming years will show whether or not the Dutch can do it!

Teat Condition in Dairy Cows

Francesca Neijenhuis (NL)

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The dairy cow's teat is the first line of defence against mastitis pathogens. The milking process may affect the teat's condition, increasing the risk of mastitis. It is well-proven that teat-ends with severe erosions or broken skin will have an increased risk of mastitis. However, more common changes in teat condition because of milking have not been related to udder health problems. The focus of this thesis was on the relationship between teat-end condition, machine milking and occurrence of mastitis. In this thesis, two types of changes of teat-end condition were distinguished: callosity rings around the orifice and machine-induced teat swelling.

First a classification system of the callosity rings around the orifice was defined: the teat-end callosity (TEC) classification system. In this system, a distinction is made between roughness of the callosity ring (TECR) and thickness of the callosity ring (TECT). Teats can be scored in 8 categories. In an experiment, it was demonstrated that TECT and TECR increased rapidly during the first 8 weeks after calving. Cow factors such as days in milk, parity, machine-on time, and teat-end shape were associated with TEC.

In order to measure machine-induced teat swelling, a methodology has been developed using ultrasound. Using this method the changes in teat tissue in relation to machine milking and the recovery time of teat tissue after milking were evaluated. Machine milking had a considerable effect on the length of the teat-canal, the width of the teat-cistern, the width of the teat-end, and the thickness of the teat-wall. 8 h after milking the teat-end width and the teat-canal length still differed from what they had been before milking. However teat-wall thickness and the teat-cistern width had recovered after 6 and 8 hours.

The TEC classification system developed was used in a 1½ year longitudinal field study on 15 farms to examine the relationship between TEC and the incidence of clinical mastitis. From that study it was clear that clinical mastitis does have a relationship with TEC. The relationship between TEC and mastitis was different for different mastitis pathogens. *Escherichia coli* mastitis occurred more in quarters where teats had less TEC. Specific pathogens differ in their opportunistic use of orifice dysfunction to multiply or to enter the teat canal. Pointed teat-ends had higher TECT and TECR than flat or inverted teat-ends and TECT increased with a higher milk yield at peak production.

The same longitudinal data set was used to focus on TEC as a risk condition for mastitis. Teats with a thin and smooth TEC ring showed the lowest incidence risk of clinical mastitis. They can be regarded as physiologically normal teats. An increase in the risk of clinical mastitis was observed when thickness and/or roughness of TEC increased. This is caused by the teat canals being less tightly closed after milking, by an excessive turnover of keratin, or the harbouring of pathogens in TEC. An increase in TECT and TECR can be used as an early warning signal for enhanced risk of clinical mastitis.

Quarters without any callosity ring also showed an increased incidence risk of clinical mastitis during the next month. The higher risk of IMI in teats without a callosity ring may

be caused by a decreased rate of keratin regeneration in the teat canal.

To evaluate TEC in the field, a simplified 4-category scoring system is suggested and used in an observational study on 200 dairy farms. Cows that have an increased risk of clinical mastitis due to more TEC build up because of milking were the subject of interest in this study. For this purpose, categories of TEC that gave an increased risk on mastitis were combined into one group: %ROUGH. Variation in %ROUGH between farms is explained by cow factors such as teat-end shape and machine-on time and milking machine factors such as the liner and the vacuum.

The overall conclusion of this thesis is that a healthy teat of a dairy cow has a good balance between the physiological reaction to machine milking and maintaining its first line of defence mechanism against invading mastitis pathogens. Increasing rates of IMI were related to one or more of the following: a high degree of machine-induced swelling, a high level of TECT, a high level of TECR and the absence of TEC. Pathways through which these machine-induced changes lowered the resistance of the teat to bacterial invasion are the openness of the teat canal, harbouring of pathogens in TEC and significantly increased or decreased level of keratin regeneration rate. Part of the impaired reaction of the teat to machine milking may lie in the peak milk flow rate.

Suggestions are made to adjust the characteristics of machine milking to the milk flow profile of an individual cow. This can minimize machine-induced teat condition problems.

Teat condition changes can be used as an early warning signal for enhanced risk of clinical mastitis. Classification of teat condition is an essential tool in milking machine research and a useful monitoring tool of the quality of milking in the field. Protocols for systematic evaluation of teat condition are available.

Mastitis in dairy production: Current knowledge and future solutions

Edited by H. Hogeveen

Worldwide, mastitis is still one of the most important diseases in the dairy sector. Mastitis being a multifactorial disease, caused by multiple pathogens, its control remains a difficult issue. Mastitis not only affects the health of milk-producing animals, having consequences for the profitability of dairy farms, it also affects the welfare of the animals. Moreover, mastitis negatively influences the quality of the milk with consequences for the dairy processing industry. In other words: mastitis affects a large part of the dairy production chain.

Thanks to on-going scientific effort, insight into mastitis in the context of increasingly complex farming systems is improving. This insight leads to better methods to control mastitis either by prevention or by adequate measures (for example, therapy) when a cow (or goat or sheep) gets mastitis.

This book reflects current knowledge on mastitis from all over the world as it was presented during the 4th IDF International Mastitis Conference, held in June 2005 in Maastricht, the Netherlands. Moreover, the 115 oral presentations and the 13 keynote presentations reflect not only **current knowledge** of mastitis control but also give ideas for **future solutions** for control measures.

June 2005

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MASTITIS NEWSLETTER No 26

ABSTRACT

IDF work on Animal health:

Mastitis remains a major preoccupation for IDF but attention is now being given to other infectious diseases (BSE, TB, FMD) and production diseases, starting with lameness. IDF's contacts with the World Organisation for Animal Health (OIE) have developed strongly, OIE participating in IDF work and events and IDF in OIE's.

L Kulkas, Chair

Somatic cell counts

The International standard methods for somatic cell counting (IDF 148/ISO 13 366) is being revised. The Draft International Standard text is circulated for comment in 2005.

H van den Bijgaard et al

Research communications and university theses

Impact of changing dry cow strategies *E A Berry*

Mastitis control in organic herds *L Echevarria et al* (English/français)

Escherichia coli mastitis – bacterial factors and host response *T Lehtolainen*

Diagnostic potential of the CMT to detect sub-clinical mastitis *J Hamann et al*

Role of lactoferrin in treatment of bovine mastitis *T Kutila*

Monitoring bovine mastitis in Finland – 1988-2001 *A Pitkälä et al*

Ten years of inter-comparisons on counting somatic cells in raw milk *E-H Ubben & K Knapstein*

Microscopic counting of somatic cells in milk *E-H Ubben et al*

Influence of storage conditions of antibiotics on excretion time in milk *K Knapstein et al*

Five year project to reduce mastitis in the Netherlands *T Lam*

Teat condition in dairy cows *F Neijenhuis*

International symposia

Automatic milking – a better understanding, Lelystad (NL), 2004

4th IDF International Mastitis Conference, Maastricht (NL), June 2005 – *All there is to learn about mastitis!*

Continuity of science, communication and wisdom – an appreciation of Woody Pankey

M W Woolford

Publications on mastitis

"Mastitis in dairy production: Current knowledge and future solutions"

Ed: *H Hoogeveen*

In English (un article également en français)

37 pages

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All papers should be written in English.

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 - * If the publication is a book, names of the publishers, city or town, and the names and initials of the editors;
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 - * Page number or number of pages, and date.

Example: 1 Singh, H. & Creamer, L.K. Aggregation & dissociation of milk protein complexes in heated reconstituted skim milks. J. Food Sci. 56:238-246 (1991).

Example: 2 Walstra, P. The role of proteins in the stabilization of emulsions. In: G.O. Phillips, D.J. Wedlock & P.A. Williams (Editors), Gums & Stabilizers in the Food Industry - 4. IRL Press, Oxford (1988).

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IDF's conventions on spelling and editing should be observed. See Annex 1.

ANNEX 1 IDF CONVENTIONS ON SPELLING AND EDITING

In the case of native English speakers the author's national conventions (British, American etc.) are respected for spelling, grammar etc. but errors will be corrected and explanation given where confusion might arise, for example, in the case of units with differing values (gallon) or words with significantly different meanings (billion).

".....	Usually double quotes and not single quotes
? !.....	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
microorganisms.....	Without a hyphen
Infra-red	With a hyphen
et al.....	Not underlined nor italic
e.g., i.e.,... ..	Spelled out in English - for example, that is
litre.....	Not liter unless the author is American
ml, mg,... ..	Space between number and ml, mg,...
skim milk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate.....	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC <u>International</u>	Not AOACI
programme	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma.....	in Standards (only) in both languages (as agreed by ISO)
No space between figure and % - i.e. 6%, etc.	
Milkfat.....	One word
USA, UK, GB.....	No stops
Figure	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours.....	ø h
second	ø s
litre.....	ø l
the Netherlands	

Where two or more authors are involved with a text, both names are given on one line, followed by their affiliations, as footnotes

for example A.A. Uthar¹ & B. Prof²

¹ University of

² Danish Dairy Board

IDF does not spell out international organizations

