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MASTITIS NEWSLETTER 21

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Editor: Prof. Dr M. Schällibaum

Mastitis Newsletter aims mainly at disseminating succinct information on the work, plans and achievements of an IDF Group of Experts dealing with Bovine Mastitis (Group A2)*, but also includes information available to the Group from other sources such as the National Mastitis Council (NMC) in the USA. Group A2 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 Group A2. 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), General Secretariat, Square Vergote 41, 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 Group A2**

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Invited member: G. Kalatzopoulos (GR) (for Group A7).

CONTENTS

* *Summaries of papers presented at the conference on the occasion of the meeting of Commission A during the IDF Annual Sessions in Vienna, September 1995.*

REPORT OF THE IDF GROUP OF EXPERTS ON MASTITIS

The IDF Group of Experts on mastitis (Group A2) met on 14 November 1995 at IDF Headquarters in Brussels and on 24 April 1996 in Liebefeld-Bern, Switzerland. Both meetings were well attended by members from Europe and North America. The Subgroup on Machine Milking and Mastitis met on the day prior to each of these meetings and reported their activities to the main Group A2.

The Group A2 sponsored a conference on the "Significance of Somatic Cells in Milk" at the 1995 IDF Annual Sessions in Vienna, Austria, under the chairmanship of Prof. W. Heeschen (DE). Five papers were presented and were as follows: Mastitis: the disease under aspects of milk quality and hygiene – Prof. W. Heeschen; Quality assurance in measuring and testing for somatic cells in milk – Prof. M. Schällibaum (CH); Somatic cells in milk and their significance for milk processing (technology) – Dr A. Zecconi (IT); Somatic cells: factors of influence and practical measures to keep a physiological level – Prof. J. Hamann (DE); Standards for somatic cells in milk: Physiological and regulatory – Prof. L. Smith (US). Readers will note that condensed versions of these papers have been published in this issue of the Newsletter.

A small group under the leadership of Dr O. Østerås (NO) has produced a document entitled "Recommendations for the presentation of mastitis related data". The document was approved by Group A2 and has been submitted to the Permanent Committee of Commission A for publication approval as an IDF Bulletin. The document makes recommendations on appropriate methods for summarization and presentation of somatic cell count and clinical mastitis data. A condensed version of the recommendations will precede the main text.

The Machine Milking and Mastitis Subgroup, A2D, met twice under the chairmanship of Prof. J. Hamann (DE). The Subgroup has prepared a document entitled "Dynamic testing of machine milking" and the document was approved for publication after several corrections and changes were made. There was consensus that the document title should be changed to "Guidelines for evaluation of the milking process". A second document, "Evaluation of the electrical conductivity of milk as a mastitis indicator", was approved for publication after inclusion of some recently published information and the addition of comments regarding potential future application of the technology.

There was major discussion at the April 1996 meeting in Switzerland regarding the future of Subgroup A2D, as well as Subgroup A2B (Cell Counting). There was consensus that both Subgroups had basically completed their terms of reference and that both groups should be disbanded. Several new items have been suggested as valuable areas for future action or efforts. The Group A2 plans to meet on 18 November 1996 in Brussels for a day-long brainstorming session on future objectives for A2. Group A2 anticipates that new subgroups or working groups will result from these efforts. Topics for discussion include: the Mastitis Control Questionnaire; consideration of future ring trials; teat stimulation and its relation to infection risk; teat skin condition and new infection risk; emerging technologies and their impact on udder health; new measures for mastitis and milk quality; and available techniques for detection of udder pathogens with risk for humans.

Edition No. 20 of the IDF Mastitis Newsletter was published in September 1995 and contained a wide range of articles relating to mastitis control and the production of quality milk. The Newsletter is edited by Prof. M. Schallibaum (CH). The Mastitis Research Index prepared by Prof. H. Saloniemi (FI) continues and a new edition was published in January 1996. The next edition is scheduled for 1998 and will be available as both hard copy and as an Internet version.

Other topics discussed by Group A2 during the year included: antibiotic residues and microbial resistance; application of mastitis vaccines; use of teat dips and the practice of predipping , and homeopathic remedies. Regular updates on the current activities and future meetings of the US National Mastitis Council were provided by Prof. L. Smith (US). A joint NMC/IDF half-day seminar on the topic of "Milk production: hazards and risks from microbial pathogens and chemical residues" is scheduled for February 1997 and will be coordinated by Prof. L. Smith (US). A second joint IDF/NMC seminar is planned for the spring A2 meeting in Guelph, Ontario (CA). The title is "Environmental streptococci and mastitis control". This effort will be coordinated by Prof. K. Leslie (CA). One change in membership occurred and involved the elevation of Prof. J. Hamann (DE) from an observer to an official delegate from Germany.

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INTEGRATED DETECTION SYSTEMS FOR ANTIMICROBIALS IN MILK: *THE IDF APPROACH*

1 INTRODUCTION

Antimicrobials in milk form a "hazard", which is defined as an "undesired property" of a food. A hazard can lead to a "risk", which is the probability that an undesired event can occur. Minimization of hazards and risks is the dominant task.

Testing of milk for the presence of antimicrobials (antiinfectives) and inhibitors is based on two different approaches to ensure toxicological and technological safety.

Concerning detection methods it has to be accepted that there is no single test available covering all technological and toxicological needs. The complex situation is illustrated in Tables 1 and 2.

The maximum residue limits (MRLs) and the safe/tolerance level are fixed differently by Codex Alimentarius, EU and the Food and Drug Administration (FDA) in the USA (Table 1). Microbiological inhibitor tests do detect antimicrobials in MRL concentrations in a limited number of cases (Table 2). It is therefore necessary to combine different methods in a system, forming a so-called "integrated system", in which a number of different methods are applied depending on the targets or objectives of the analysis.

In the following the principles of such an integrated system are described.

Table 1: Maximum Residue Limits (MRLs) and "safe/tolerance" levels for residues of antimicrobials in milk ((g/kg)

Group	MRL Codex	MRL EU (1)	Safe/ tolerance (2)	Group	MRL Codex	MRL EU (1)	Safe/ tolerance (2)
β-Lactam antibiotics				**Macrolides**			
Penicillin	4	4 (5)	5/0	Erythromycin		40 (6)	50/0
Ampicillin		4 (5)	10/10	Spiramycin	100	200 (5)	
Amoxicillin		4 (5)	10/10	Tylosin		50 (6)	50/50
Cloxacillin		30 (5)	10/10				
Dicloxacillin		30 (5)		**Aminoglycosides**			
Oxacillin		30 (5)		Gentamicin	100 (9)	30/0	
Ceftiofur	100	100 (6)	50 (7)/1000 (8)	Neomycin	500 (9)	150/150	
Cephapirin			20/20	DH Streptomycin	200 (9)	125/0	
Tetracyclines (3)		100 (6)		**Others**			
Chlortetracycline			30/0	Dapsone		0 (5)	
Oxytetracycline	100		30/0	Chloramphenicol		0 (5)	0/0
Tetracycline			80/0	Novobiocin		100/100	
				Spectinomycin	200 (9)	200 (6)	30/0
Sulfonamides (3)		100 (6)		Trimethoprim		50 (6)	
Sulfadimidine (4)	25		10/0				
Sulfadimethoxine			10/10				
Sulfamerazine			10/0				
Sulfathiazole			10/0				
Sulfadiazine			10/0				

(1) EU regulation 675/92, 3093/92, 2901/93, 3426/93, 1430/94, 2703/94, 3059/94, 1442/95; (2) CFR 21 and CVM correspondence; (3) All substances belonging to the group; (4) Sulfamethazine; (5) Final; (6) Preliminary; (7) Parent drug; (8) Total parent and metabolite; (9) Compounds on agenda.

Table 2: Detection of antimicrobials in Maximum Residue Limit (MRL) concentrations (µg/kg) with microbiological inhibitor tests

Compound	MRL EU	BR Blue Star	Delvo SP	*B. cereus*
Penicillin	4	yes	yes	–
Ampicillin	4	yes	yes	–
Amoxicillin	4	yes	yes	–
Cloxacillin	30	no	yes	–
Dicloxacillin	30	no	yes	–
Oxacillin	30	(91%)	yes	–
Ceftiofur	100	yes	yes	–
Sulfadimidine	100	(57%)	(85%)	–
Sulfadimethoxine	100	yes	yes	–
Dapsone (2 µg/kg)*	0	yes	(33%)	–
Trimethoprim	50	no	no	–
Tetracycline	100	no	no	yes
Oxytetracycline	100	no	no	yes
Chlortetracycline	100	no	no	yes
Tylosin	50	yes	(85%)	–
Spiramycin	150	no	no	–

** Preliminary results, further confirmation necessary.*

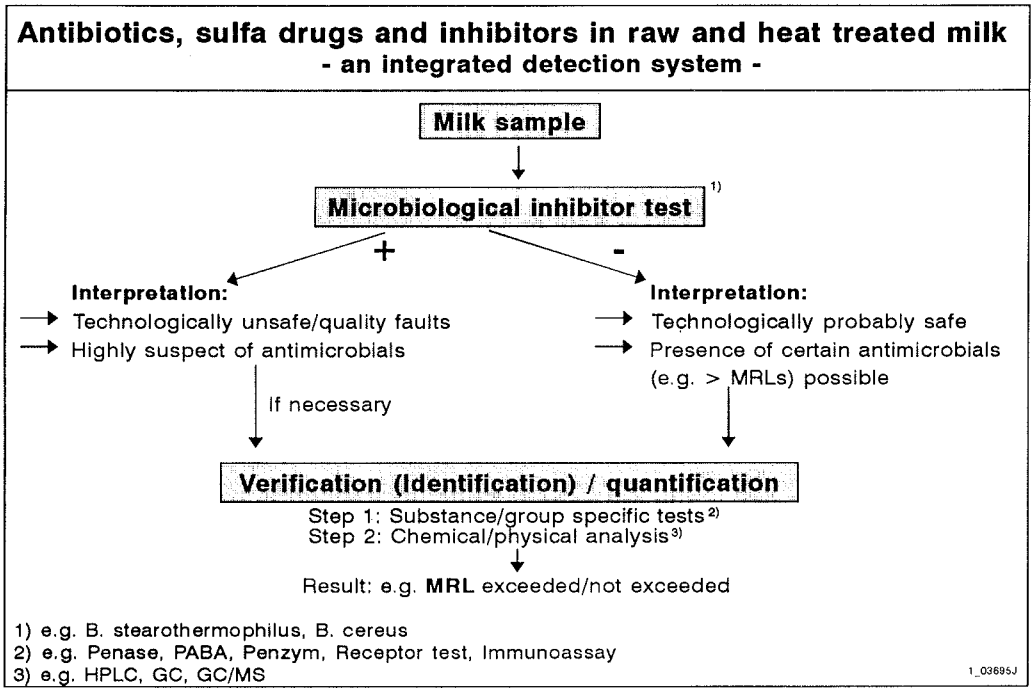

Figure 1

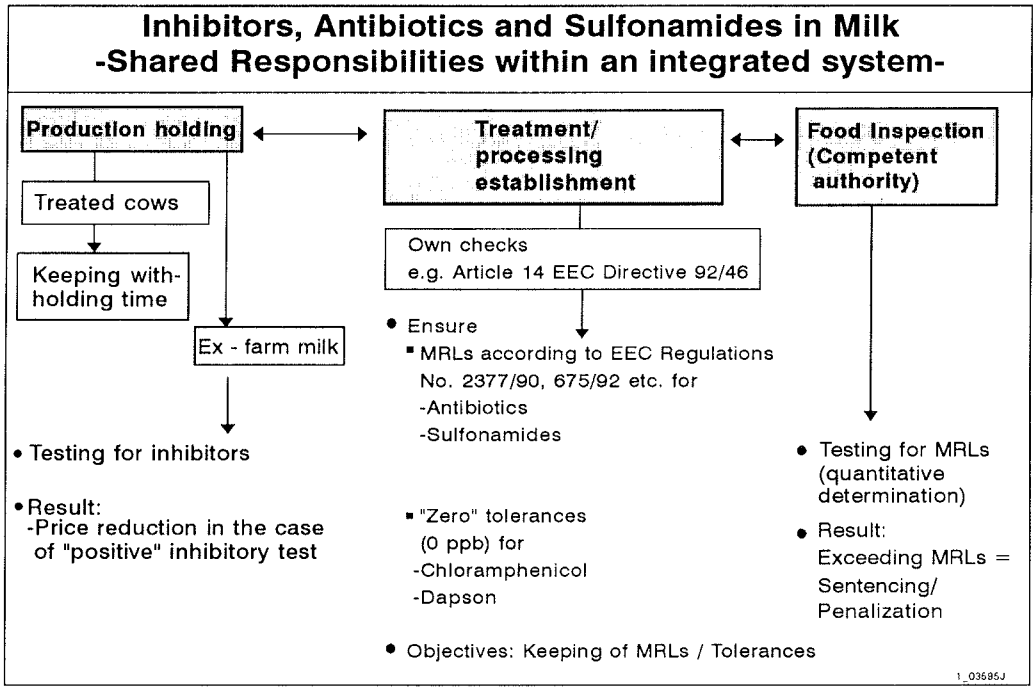

Figure 2

2 PRINCIPLES OF AN INTEGRATED DETECTION SYSTEM FOR ANTIMICROBIALS AND INHIBITORS

An integrated detection system for antimicrobials and inhibitors in raw and heat-treated milk has to distinguish between

- aspects of the method applied, and
- the responsibilities of milk producers, treatment/processing establishment and food inspection within this system.

The method-oriented integrated detection system is shown in Figure 1.

Positive microbiological inhibitor tests lead to the interpretation that the milk could be technologically unsafe and are highly suspect of the presence of antimicrobials. A negative microbiological inhibitor test result can not exclude the presence of certain antimicrobials, which are not detectable by test organisms commonly used. The shared responsibilities within an integrated system are explained as an example in Figure 2.

The production holding or the farmer has to keep the withholding time. The ex-farm-milk must be regularly tested for inhibitors, using a microbiological inhibitor test. The treatment/processing establishment has to make sure that MRLs or other tolerances are not exceeded. Therefore it is proposed to have regular tests for inhibitors on the tanker level using a microbiological inhibitor test and, in addition, tests for a number of other compounds like chloramphenicol, dapsone and tetracyclines, which are not

detectable with the microbiological inhibitor test used. The food inspection (competent authority) has to identify antimicrobial residues and determine whether or not MRLs are exceeded. Of course, this system can not clearly be separated for the levels of production, processing or food inspection. There are many interactions, and the combination of the three "hurdles" should give maximum technological and toxicological safety. The system described in Figure 2 is explained further in Figures 3 and 4, which show how the system is run under practical conditions.

3 EVALUATION OF METHODS WITHIN AN INTEGRATED DETECTION SYSTEM

The use of different methods in different countries requires the development of test-kit performance testing programmes to ensure the detection of concentrations of residues below

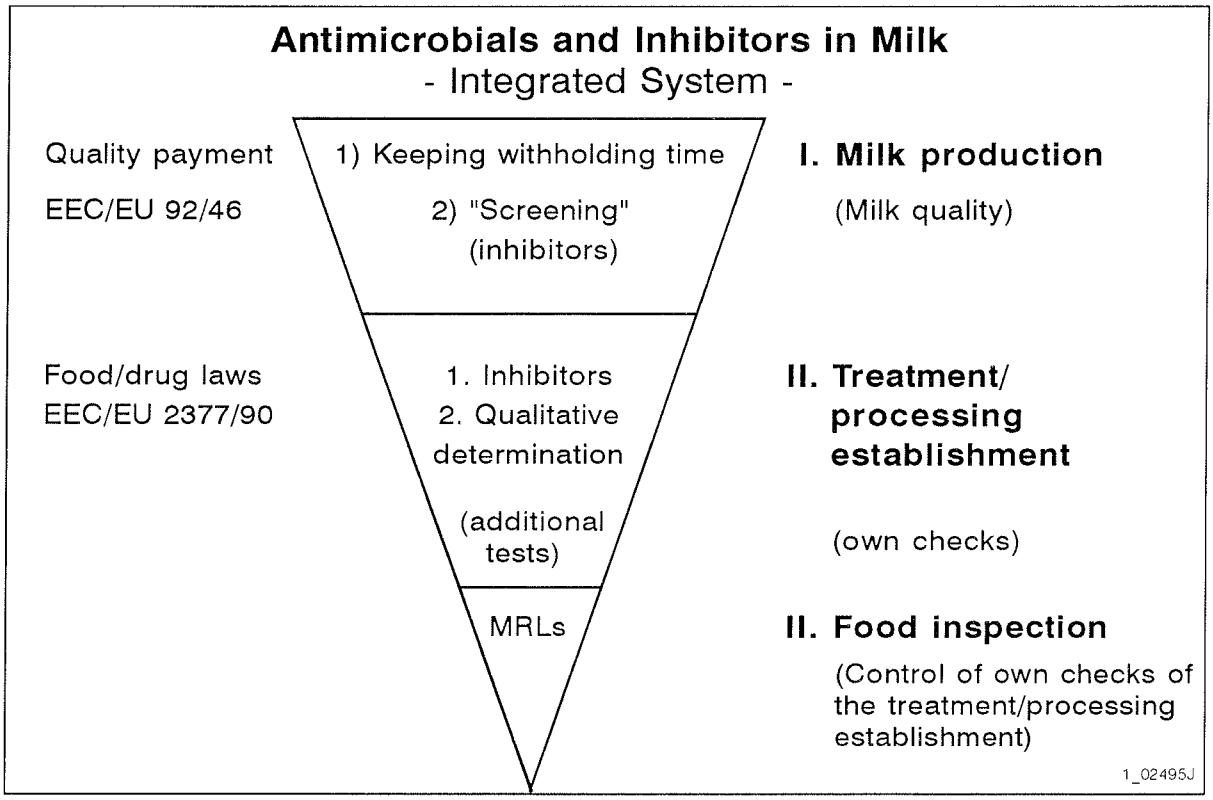

Figure 3

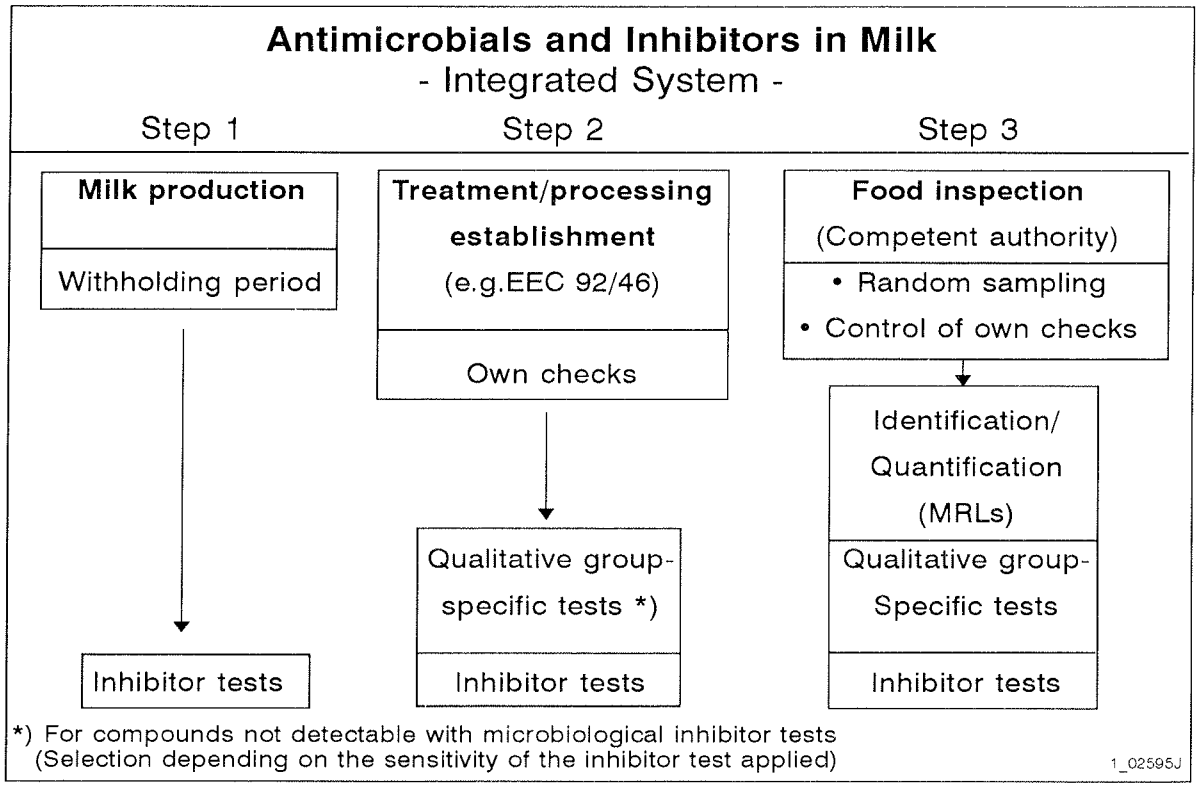

Figure 4

the MRLs or the safe levels. The different performances of tests used worldwide for the detection of antibiotics and sulfa drugs show that the MRL for tetracycline is detected only by certain tests.

Most often the detection pattern of a test which includes the spectrum of antimicrobials detected and the respective detection sensitivities is the crucial characteristic at a given combination of test conditions. Figures 5 and 6 are an attempt to visualize such detection patterns with regard to a set of antimicrobials and in this model 4 test kits. For each antimicrobial in the set a standardized MRL scale is provided. Standardized scale means scaling by n-fold MRL concentrations. The ideal test should detect 1 x MRL of all antimicrobials of concern which corresponds to the bold middle circle in the Figure. The more sensitive with respect to MRL requirement an antimicrobial can be detected the further is the distance from the centre on the standardized MRL scale and vice versa. A picture of the detection pattern of a test results from connecting the detection limits of the set of antimicrobials. The resulting area represents the detection pattern of a test. The detection patterns of different tests can easily be compared. In the same way the test conditions within a given test – for example different batches – can be compared visually.

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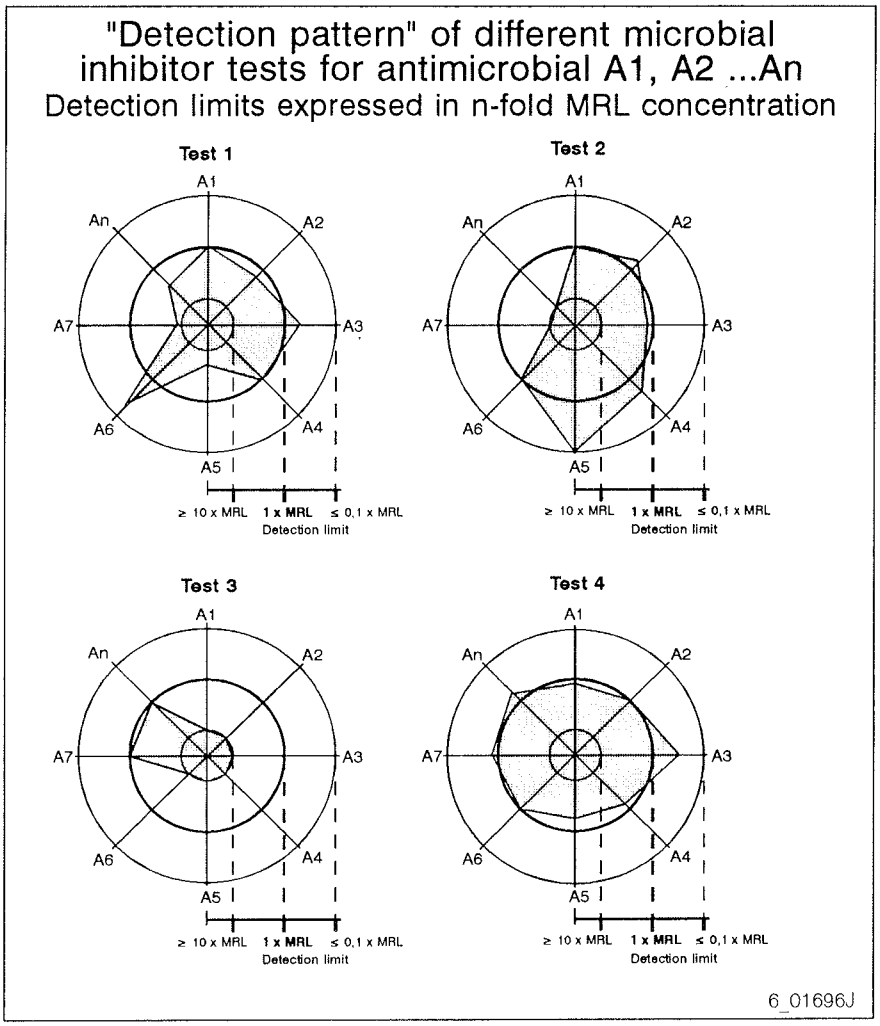

Figure 5

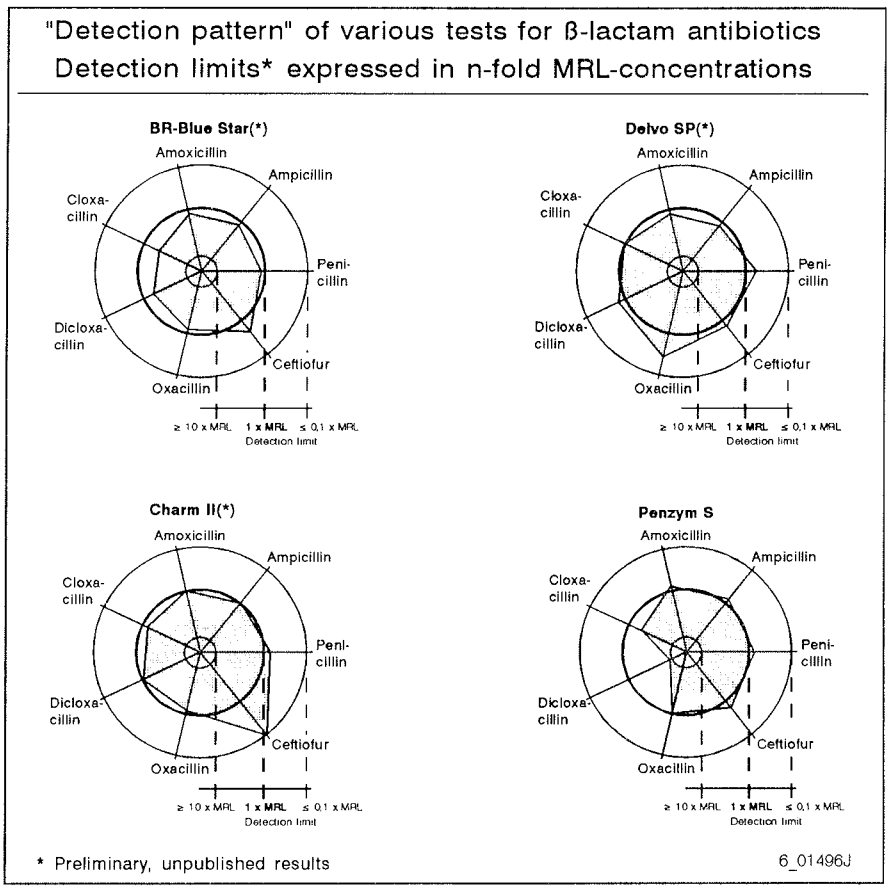

Figure 6

Research Communications

STANDARDS FOR SOMATIC CELLS IN MILK: PHYSIOLOGICAL AND REGULATORY

ABSTRACT

Somatic cell counts in milk from individual cows of <200 000/ml are almost always considered physiologically normal while cell counts >300 000/ml are generally considered indicative of the presence of inflammation. A somatic cell count value of 250 000/ml is suggested by IDF as dividing normal milk from milk from mastitic glands. Somatic cell counts of herd bulk milk are used as indicators of milk quality and mastitis control, and indirectly as an indicator of the hygienic production of milk. Somatic cell count standards for legally acceptable milk vary from 400 000/ml in the European Union countries to 750 000/ml in the United States. Little evidence exists that would indicate that elevated somatic cell counts are a direct risk to human health. Negative effects on milk quality can be detected at cell counts as low as 250 000/ml. Standards for bulk milk should be based on scientific principles rather than political and trade issues.

1 INTRODUCTION

Demands by consumers and processors for safe, high quality dairy products together with pressure from international markets for products with documented high quality, are the major factors driving the standards for somatic cell counts (SCC) in milk [1–3]. All major dairy producing countries use SCC as a measure of milk quality.

Milk SCC are a monitor of inflammation of the mammary gland of cows and there is considerable agreement among research workers that the primary cause of inflammation is bacterial infection [4]. Good udder health is essential for quality milk production and SCC is the most widely accepted criterion for indicating the udder health status of individual cows within dairy herds, milk cooperatives, regions, and countries [1, 5, 6].

At the cow level, SCC are used to determine if the individual cow or quarter is mastitic and the likelihood that the individual cow or quarter is infected [6]. Intramammary infection status can only be proven by bacteriological culture of aseptically obtained milk samples. The SCC of the commingled milk from the entire herd, or bulk tank milk somatic cell count (BTSCC) is a measure of the amount of mammary disease or mastitis in the dairy herd [7, 8]. In addition, BTSCC are related to a number of product quality issues such as cheese yields, shelf life, appearance, flavor, and odor [9].

2 PHYSIOLOGICAL STANDARDS

Several comprehensive reviews or individual studies over the past 20 years have addressed issues surrounding SCC, their variation, and the potential use of SCC for monitoring milk quality [4–8, 10]. Clearly the major factor affecting SCC is an infection of the mammary gland and this holds true at the quarter, cow, or bulk tank level [4]. Other factors, such as lactation number, stage of lactation, and season of the year, have only minor influences by comparison.

The vast majority of studies would suggest that cows with SCC of less than 200 000/ml are not likely to be infected with major mastitis pathogens, while cows with SCC of 300 000/ml or greater are very likely to be infected [4, 6, 7]. Depending upon the study, the threshold SCC indicative of inflammation or mastitis has been set at 200 000/ml, 250 000/ml or 283 000/ml. A SCC reference value of 250 000/ml would appear to be a useful standard indicating that milk from individual cows is either mastitic (250 000/ml or above) or physiologically normal (less than 250 000/ml) [4–7]. Support for this value is also in IDF Bulletin No. 211 [10].

The SCC value of 250 000/ml is used to indicate the presence or absence of inflammation or mastitis. While statistically the value is also useful as an indicator of the presence or absence of intramammary infection, a small percentage of cows truly infected with major pathogens will have cell counts less than 250 000/ml [4, 7]. In addition, some cows infected with the minor pathogens, and particularly the coagulase negative staphylococci, will have SCC exceeding 250 000/ml [11]. Also of importance is that cell numbers in milk are not static but part of a dynamic process regardless of the infection status [4]. Given the dynamic nature of SCC in mammary glands, interpretation of SCC based on a single sample is subject to considerable misdiagnosis or misclassification.

Bulk milk SCC have three broad uses. They are used to monitor the prevalence of mastitis in dairy herds, as an indicator of raw milk quality to processors, and as a more general indicator of the hygienic conditions of milk production on farms [1, 2, 5].

Bulk tank SCC are a function of the percentage of quarters infected by major pathogens in a dairy herd. Eberhart et al. [7] reported a linear relationship between BTSCC and the percent quarters infected with major pathogens. The relationship was described by the equation $Y = 3.3X - 0.42$, where Y = BTSCC in 100 000 cells/ml, and X = % quarters infected by major pathogens. Based on these data, the percent quarters infected would be 6.2, 12.8, 24.3, and 32.6 for BTSCC of 200 000, 400 000, 750 000, and 1 000 000/ml, respectively. In addition, BTSCC were negatively related to average daily milk yield and fat yield.

BTSCC primarily measure subclinical mastitis and poorly monitor clinical mastitis in dairy herds [4, 12, 13]. Given the fundamental differences in epidemiology of mastitis pathogens, BTSCC do a better job monitoring mastitis caused by contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* than mastitis caused by the environmental pathogens such as *Streptococcus uberis* and *Escherichia coli* [13]. The latter are frequent causes of clinical mastitis, and high SCC milk from clinical cows generally is withheld from the bulk tank. The experience in the United States is that many herds consistently producing

low BTSCC milk (<250 000/ml) can still experience undesirable incidence of clinical mastitis [12]. No evidence is available indicating that clinical mastitis will automatically increase if a herd produces low SCC milk.

Research of the past 10 years has established a clear relationship between BTSCC and the quality of the milk for processing purposes. Much of the reduced quality associated with mastitic milk is due to the enzyme plasmin [9, 14, 15]. Plasmin is found in both blood and milk, and increases in milk during inflammation of the mammary gland. Plasmin can cause extensive damage to milk casein in the udder prior to milk removal. As BTSCC increase to 500 000 cells/ml and above, the processing properties of milk are diminished. Diminished cheese yields have been reported as SCC increase from 100 000/ml to 500 000/ml and significant quality effects may be noted at SCC as low as 250 000/ml [16]. All producers should understand that the quality of raw milk can not be improved once it leaves the farm and there is increasing demand from processors for low SCC milk.

Bulk tank SCC are also used as an indicator of hygienic conditions of milk production [2]. In general, the hygienic conditions on farms producing low SCC milk are more desirable than conditions on farms producing high SCC milk. The relationship between BTSCC and percent quarters infected would suggest a reduced probability of finding potential human pathogens in low SCC milk. Such pathogens include: *Staphylococcus aureus, Escherichia coli, Campylobacter jejuni, Yersinia enterocolitica, Listeria monocytogenes, Salmonella* spp. and *Clostridia* spp.

3 REGULATORY STANDARDS

As a result of the relationships described above, BTSCC are used: (1) to evaluate mastitis control in dairy herds; (2) in payment schemes for milk; and (3) by regulatory agencies as a monitor of hygienic production and milk safety. Regulatory agencies in the various countries have established upper SCC limits for raw milk. The European Union has imposed a SCC limit of 400 000/ml [2]. The SCC limit is currently 500 000/ml in Canada and 750 000 cells/ml in the United States [1]. The argument for the higher upper limit in the US has centered around the concept that no direct human health risk can be attributed to SCC of greater than 400 000 cells/ml. The lower limit in the European Union may be a function of greater use of raw milk and raw milk products than occurs in the United States and greater emphasis on milk quality for processing purposes.

Various milk cooperatives and processors in the US have encouraged the production of low SCC milk by offering producers incentives for low SCC milk rather than penalties for high SCC milk. Regardless of the upper limit of SCC imposed by regulatory agencies or the monetary incentives for low SCC milk, the goal of a small percentage of producers will always be to remain legal rather than the production of low SCC high quality milk. Lower standards may be necessary to persuade such producers to produce high quality milk. Lower standards may be more difficult for small herds to achieve consistently as a single cow in a small herd can have a major impact on BTSCC [17]. Conversely, there is considerable dilution of the single cow in large herds.

As a result of increasing world trade in milk and milk products there is need to establish a world standard for SCC. Mastitis can not be eradicated from dairy herds and zero mastitis in dairy herds is not a realistic goal. The relationship between BTSCC and percent quarters infected as published by Eberhart et al. [7] suggests that 12.8% of quarters would be infected at a BTSCC of 400 000/ml and 24.3% of quarters infected at a BTSCC of 750 000/ml. Obviously, substantial amounts of mastitis remain even at a SCC of 400 000/ml, and a herd at 750 000/ml has a clear and serious mastitis problem resulting in a considerable loss of money to the producer. The technology to produce milk consistently and economically with a BTSCC of less than 400 000/ml is available in all major dairy countries of the world. These data argue in favor of the European Union SCC standard of 400 000/ml as the basis of international trade in safe, high quality milk and milk products.

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SOMATIC CELLS: FACTORS OF INFLUENCE AND PRACTICAL MEASURES TO KEEP A PHYSIOLOGICAL LEVEL

1 INTRODUCTION

Successful mastitis control is of great importance with regard to food hygiene and economic aspects. Therefore, to ensure stable and physiological milk cell counts, it seems useful to evaluate the causes of milk cell fluctuations and to consider practical measures to keep the cows healthy.

2 DESCRIPTION OF A PHYSIOLOGICAL CELL COUNT LEVEL

Milk cells are somatic cells as they are derived from the macroorganism, the cow. Leucocytes as macrophages, neutrophils and lymphocytes make up the majority of the milk somatic cell count. Overall, the mean percentage of the total count in healthy glands is as follows: PMN, 15%; lymphocytes, 25%; and macrophages, 60% [1, 2]. Milk derived from healthy glands has a modal value of 20–50 000 cells/ml which results in a physiological range up to 100 000 cells [3]. Figure 1 shows the frequency distribution curve of cell counts of 3770 foremilk quarter samples with a maximum at 20 000 cells (modal value) and, if the two-fold standard deviation is used as the safety factor to define the norm, the resulting threshold for normal cell counts is about 100 000 cells/ml milk.

3 FACTORS OF INFLUENCE

Secretory and non-secretory influences are involved in the variation of cell counts. The non-secretory factors consist mainly of type of sampling, method of cell count determination, storage and transport of samples.

The most important factors of influence are of secretory nature and can be divided into three groups: (1) physiological and pharmacological factors; (2) mastitis causing factors, and (3) stress factors [4].

The somatic cell count (SCC) is high throughout the first weeks of lactation, decreases during mid-lactation, and increases at the end of lactation. The extent of lactation stage related increase in SCC depends mainly on the udder health status. Infected quarters have a much higher increase than healthy glands. The lactation number as such has no significant influence on the SCC. If older cows show a higher SCC then this is reflecting the individual mastitis history [5].

Numerous microorganisms – bacteria, moulds and yeasts – may be involved in the development of mastitis. Noxious agents like microbes and their toxins cause local haemodynamic changes resulting in exudation and emigration of cells, mostly neutrophils. Milk of diseased quarters may have up to several millions of cells/ml with >95% neutrophils

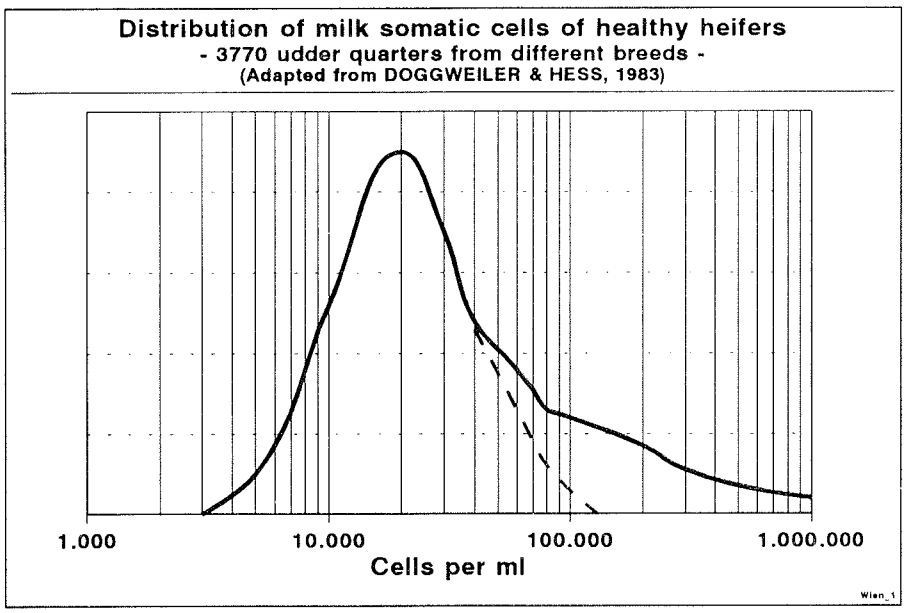

Figure 1

depending on the extent of the inflammatory process [5].

Stress factors may influence the somatic cell count either by immunosuppression, with the consequence of an increased risk for new intramammary infection (indirect effect), or by exacerbation of existing latent infections (direct effect) [6].

4 PRACTICAL MEASURES FOR PHYSIOLOGICAL CELL COUNTS

Prevention of mastitis is the key to keep the milk cell count in a physiological range. The new infection risk of the mammary gland is determined by (i) the level of exposure to pathogens, and (ii) the efficiency of the bovine defence mechanisms. All measures applied to minimize the new infection risk must be related to herd-specific conditions documented in a monitoring system. This includes that mastitis prevention is applied as a planned measure based on mastitis records including cyto-bacteriological examinations of quarter samples, and on the trend in the bulk milk cell count or preferably the cow individual cell count. Mastitis can be diagnosed only on the basis of information concerning the causative agent (different microbes) and inflammatory parameters (for example cell count, Nagase,etc.) as an expression for the degree of inflammatory reaction of the mammary gland [7].

4.1 Prevention of exposure to pathogens

Contamination

The type and extent of the contamination risk is determined by the type of mastitis pathogen (cow-associated: for example *S. aureus*; *S. agalactiae*; environment-associated: for example *E. coli*; *S. uberis*) and the particular environmental conditions. Prevention of contamination must be directed towards different aspects (Table 1).

Invasion

The nearly regular and permanent contamination of the teat end with a variety of microbes results only relatively seldom in new infections: per cow and lactation in a range between 0.2 and 2.0 infections. Obviously, cows have developed a high degree of resistance to infections in the course of their evolution. Most of this resistance lies in the teat duct as a barrier to penetration of pathogens. For the most part, growth of the microbes and diffusion are the most important principles involved in the penetration of the teat duct. A predisposing step for the penetration consists of the colonization of the teat duct by mainly contagious pathogens. Impairment of the health of the teat duct and skin of the teat are the factors most responsible for teat duct colonization. Therefore muddy housing conditions, teat tramps and milking machine induced teat damage should be avoided. Overall, a proper function of the milking technique and the regular application of a post-milking teat disinfectant containing an emollient are the methods of choice to minimize teat duct colonization and invasion.

Table 1: Practical measures to minimize the contamination risk of the bovine mammary gland (Adapted from Hamann [6])

GENERAL ASPECTS
Reduction of mastitis prevalence
Cow-related housing dimensions
Indoor climate (humidity, ventilation)
Avoidance of diarrhoea (feeding regime)
Regular hoof trimming
INTER-MILKING INTERVAL
Bedding: dry, clean, daily change, an organic material
Walking areas: dry, clean
Drying off: application of antibiotics
MILKING TIME
Teat condition: dry and clean before milking
Teat disinfection: before and after milking
Milking procedure: fast and complete evacuation
Feeding: just after milking
Milking technique: in relation to DIN/ISO NORMS

4.2 Maintenance and improvement of the bovine defence mechanisms

In addition to the question of exposure to pathogens, the success of mastitis prevention will be determined by the efficiency of both the systemic defence mechanisms of the cow and the corresponding local system in the teat end acting as the first barrier against invading microbes.

The cow performance depends on a complex interaction with many environmental factors. Finally the cow's individual ability to fit into her environment is determined by her physiological and behavioural adaptation to the actual condition. A variety of factors contribute to the homoeostasis (physiological balance) of the cow which is interacting with all fundamental functions of the macroorganism. The higher the degree of homoeostasis, the higher the available defence potential and the lower the new infection risk. High yielding cows often have metabolic disorders during early lactation periods, resulting in reduced defence potential. Therefore, a well calculated feeding regime which maintains the cow metabolically balanced is an important element as a mastitis preventive measure.

Cows have been selected mainly on yield and milkability characteristics. High yielding cows have on average shorter teats and higher milk flow rates. Both factors may contribute to an increased new infection risk. If the teats are becoming too short an effective pulsation by the machine is not possible which results in increased teat tissue impairment. Fast milking cows have a greater teat duct diameter than slow milking cows. Increased teat duct diameter offers a higher invasion risk not only during lactation but also during dry periods of cows [8]. For practical mastitis control it can be concluded that further selection of cows with extreme high peak flow rates should be avoided.

5 CONCLUSION

Prevention of new infections of the bovine mammary gland is the key to ensuring physiological milk cell count level. Related measures should keep the cow and the teat tissue in a physiological balance (homoeostasis) to provide a sufficient defence. Moreover, the level of exposure to pathogens should be reduced as much as possible.

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SOMATIC CELLS AND THEIR SIGNIFICANCE FOR MILK PROCESSING (TECHNOLOGY)

1 INTRODUCTION

Somatic cell counts (SCC) are widely used for evaluating milk quality and to define milk price. Many farmers currently consider SCC as a "pure number", a number not related to a biological phenomenon such as inflammatory process, but only as an indicator of milk value. Therefore it must be emphasized, particularly on field, that an increased SCC is the consequence of an inflammatory process due to the presence of an intramammary infection. Therefore SCC, being a direct expression of the severity of the inflammatory process, is a useful parameter to evaluate the relationship between intramammary infections and changes in milk composition.

There are three major pathways in which mastitis (and increase of SCC) influences cheese yield and composition

- by reducing milk yield;
- by reducing milk constituents used to produce cheese;
- by altering milk attitude to coagulation.

2 SOMATIC CELLS AND MILK YIELD

Losses in milk production associated with clinical mastitis are readily apparent; however, losses due to subclinical mastitis are largely underestimated, particularly by farmers. In recent years, milk yield losses associated with different levels of somatic cell counts have been estimated and the results give a clear figure on the correlation between SCC (and mastitis) and milk yield.

Besides well-known data from the USA [1], data from other countries, such as Italy, confirm the relationship between increase in SCC and decrease in milk yield [2]. Therefore it is possible to state that an increase in SCC over 100 000 cells/ml on an individual basis is associated with a progressive decrease in milk yield.

2.1 Mastitis and milk composition

Colonization of the bovine mammary gland by pathogenic bacteria results in a series of events which lead to changes in the 4 major components of milk (fat, protein, lactose and somatic cells) and the other minor components such as enzymes [3]. The changes in milk composition related to mastitis and elevated somatic cells have two general physiological explanations [4, 5]:

(a) injury of udder cells which reduces the synthesis of those milk components synthesized in the udder – typical examples are lactose and caseins;
(b) changes in permeability of membranes which permit increased leakage of materials from blood to milk.

3 MILK COMPOSITION AND SCC

3.1 Lipolysis

Looking in more detail at the biochemistry of milk and cheese, it is well know that, for dairy products, of particular importance is the influence of proteins (proteolysis) and development of rancid off-flavours through hydrolysis of fatty acids from triglycerides (lipolysis). In particular, the change in milk lipase activity could contribute to increased FFA concentration in both fresh or stored milks in mastitic milk [6]. The lipoprotein lipase is the primary enzyme responsible for lipolysis of milkfat triglycerides in milk, and the enzyme is activated by serum lipoproteins [7]. In normal bovine milk lipoprotein is associated mainly with the casein fraction; however, the source of lipoprotein lipase has not been determined. The enzyme could arise from capillary endothelium membranes or from endogenous synthesis by mammary gland secretory cells. In addition to lipoprotein activity, a number of other factors could influence lipolysis during infection [8].

3.2 Proteolysis

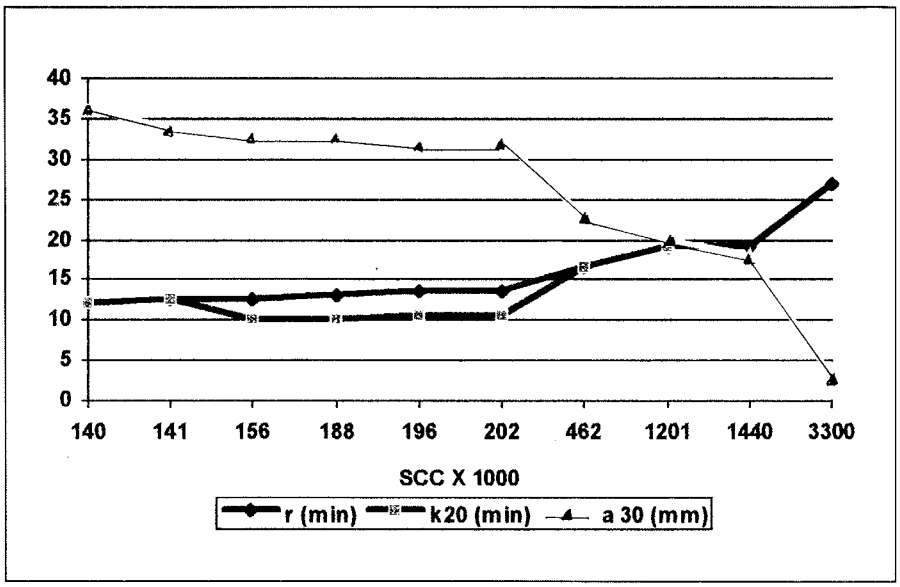
Proteolysis of milk proteins is important in dairy productions, having beneficial effects, and being necessary to generate products with desirable qualities. However, breakdown of milk proteins might adversely affect the flavour and texture of dairy products. Among the defects in dairy products associated with proteases are gelation in UHT milk, poor quality cheese, bitter peptides, casein degradation in stored caseins and viscosity changes in products containing caseins [9]. Current research suggests that an increase in proteolytic activity due to the inflammatory response may be responsible for the changes in the milk protein fraction.

Mastitic milk has more proteolytic activity than normal milk, due in part to increased proteinase plasmin, which hydrolyses plasmin. The hydrolysis alters the casein complex by decreasing the relative proportion of β-casein, which is essential for the hardening of curd caseins. If proteolytic enzymes used to coagulate milk are capable of plasminogen activation, then this could result in elevated plasmin levels in cheese and could have a significant impact on cheese flavour and texture development [10]. In mastitis milk, proteinase levels may be so high that substantial proteolysis probably occurs in the udder between synthesis and milking, and subsequently a few hours of further incubation at 37°C may be sufficient for complete hydrolysis of the caseins [11].

Politis et al. [12] investigated the relationship between several factors (stage of lactation, SCC, lactation number and season) and plasmin/plasminogen system in milk (Table 1).

The results showed that stage of lactation and SCC were of greater importance than season and lactation number in explaining variation of plasmin in milk. SCC was a more important factor than either season or stage of lactation in determining plasminogen variability in milk. Plasmin and plasminogen concentrations were increased with increasing SCC. The increase in this range was linear, where each 250 000 increase of SCC led to an increase in plasmin concentration of 0.03 mg/l [12].

Table 2: SCC and milk clotting attitude [15]



4 SOMATIC CELLS, MILK COMPOSITION AND CHEESE

4.1 Effect of milk composition on cheesemaking

In the process of cheesemaking, fat and casein are the major solids incorporated into final product. It is well recognized that cheese composition is dependent on milk composition. Because casein and fat contents and their ratio in milk influence cheese yield capacity, any factor affecting these milk components alters cheese yield capacity [13].

Politis & Ng-Kwai-Hang [14] evaluated the effect of SCC and milk composition on cheese composition and cheesemaking efficiency by the estimation of milk component losses in whey. The authors showed that SCC levels significantly affected fat, protein, total solids contents in cheese and the Moisture to Non Fat Substances (MNFS); milk containing 600 000 cells/ml gave a cheese with 0.5% less fat, 0.4% less protein and 0.9% less total solids and 0.9% more MNFS than cheese made by milk with 100 000 cells/ml.

4.2 SCC and coagulation characteristics

Milk with a SCC >500 000 has a reduced value for cheese transformation [15]. Fat takes more time to separate from the coagulum (float) and this phase is particularly important in cheesemaking (that is, Parmesan) because it is one of the ways to discard bacteria (mainly sporigenous). Milk with more than 500 000 SCC/ml has a reduced clotting strength and therefore an increase in moisture since whey is unable to be separated adequately (Table 2).

These results were confirmed, some years later, by Politis & Ng-Kwai-Hang (1988), showing that elevated SCC were associated with a significant increase in RCT; however, there was no significant difference in RCT between milk samples containing 100 000 and 500 000 SCC/ml. The same pattern has been observed for rate of firming (min K20). Meanwhile the effect on curd firmness at time of cutting (A30) showed a definite trend for lower A30 associated with increasing SCC (Table 3).

Table 1: Relative importance of factors affecting plasmin and plasminogen system in bovine milk [12]

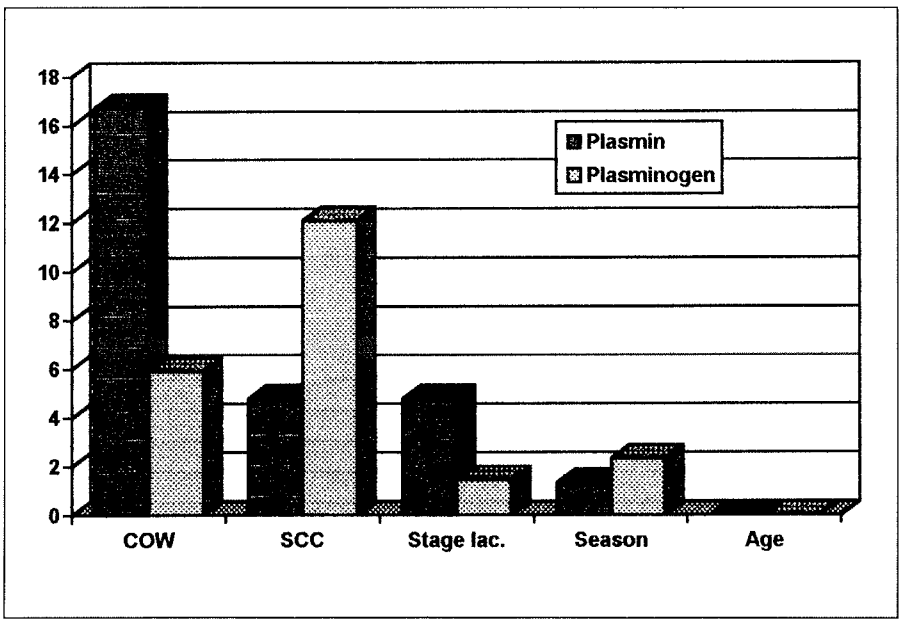
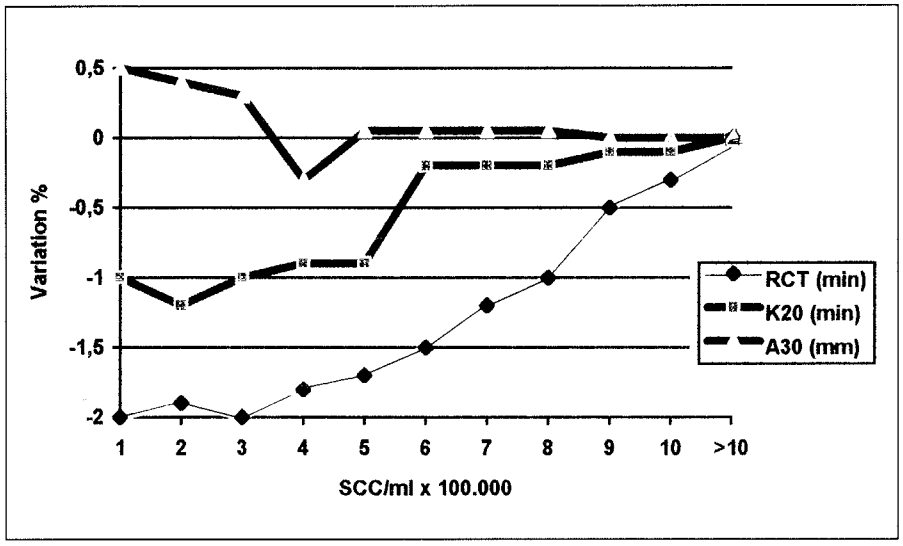


Table 3: Variability in rennet clotting time, rate of curd firming and curd firmness for different somatic cell counts (Politis & Ng-Kwai-Hang, 1988)



4.3 Cheese yield efficiency

The importance of SCC in cheesemaking can be evaluated by considering the impact of increasing milk SCC on cheese yield. This relationship seems to be not linear, most of the decrease in cheese yield due to SCC seems to occur when milk SCC change from <100 000 to >100 000 cells/ml. The total decrease in cheese yield efficiency due to the combination of SCC and milk age observed in the study of Barbano et al. [16] was about 2.56%. Large changes in milk quality occur in milk from very low SCC levels to levels >100 000 cells/ml. On an individual cow basis, further increases in milk SCC up to 1 300 000 cells/ml do not make substantial changes in milk quality given constant milking intervals and constant postmilking handling conditions. Politis & Ng-Kwai-Hang [13] confirmed that a considerable amount of variation in the adjusted cheese yield can be explained by variation of SCC. The SCC influenced both the adjusted yield and the yielding efficiency. Elevated SCC are associated with the production of high moisture cheese, and an increase of SCC from 100 000 to 900 000 was associated with an 11% decrease in yield efficiency.

This decrease must be due to reasons other than the lower casein and fat contents of milk that have a negative influence on cheese yield. In the conversion of milk to cheese, casein micelles aggregate to form an open network that entraps the whey phase. More specifically, the significant reduction in the β-casein associated with elevated SCC will result in decreased curd tension at the time of cutting. Curd tension affects cheese yield by influencing the losses of fat and casein in the whey [13].

4.4 Why control mastitis and decrease SCC ?

There is clear evidence that mastitis control and therefore a reduction of SCC means an increase in milk quality and yield. On the other hand, a mastitis control programme has its own costs in terms of investment and labour.

To give some estimation on the positive cost/benefit balance of a mastitis control programme a relatively small Italian province (Modena, typical area where cheesemaking is predominant) has been taken as an example. In this province about 50 000 cows are bred and most of the milk production is devoted to *Parmigiano Reggiano* production. Data in Table 4 estimated the losses due to SCC in Modena province, regarding 18 000 cows which are followed by the local breeders association [17].

Hypothesizing a reduction of SCC in the 2nd class (282–565) so that all the cows in this class come into the 1st class, we will get an improvement of cheese yield (Table 5).

With this simulation the total amount of cheese produced

Table 4: SCC and cheese yield in Modena province [17]

SCC x 1000	Cows	%	Casein on TP	% milk casein	Casein yield/ cow 100 kg	Cheese yield (PR)/ cow 100 kg	Total cheese yield 100 kg
0–282	11 652	62.3	0.77	2.40	1.51	4.07	47 388
282–565	3508	18.8	0.75	2.34	1.47	3.96	13 896
566–1130	2287	12.2	0.71	2.22	1.39	3.75	8576
>1130	1252	6.7	0.69	2.15	1.35	3.64	4562

Table 5: SCC and cheese yield in Modena province (cows in SCC class 282–565 moved to lower class) [17]

SCC x 1000	Cows	%	Casein on TP	% milk casein	Casein yield/ cow 100 kg	Cheese yield (PR)/ cow 100 kg	Total cheese yield 100 kg
0–282	15 160	81.1	0.77	2.40	1.51	4.07	61 656
283–565	0						
566–1130	2287	12.2	0.71	2.22	1.39	3.75	8576
>1130	1252	6.7	0.69	2.15	1.35	3.64	4562

increased from 7440 ton to 7480 ton. It would mean an increase of 40 000 kg of Parmesan, which at 7 ECU/kg (1993 farm price) is a net benefit of about 280 000 ECU (price at producer).

This amount of money regards only the benefit of increased cheese yield and has to be added to the increased quality and the decrease in health problems at farm level.

5 CONCLUSIONS

The production of milk with low SCC must be the target for any producer. The achievement of this target is justified economically, because low SCC means higher milk yield and quality and therefore higher cheese quality and yield. Data presented over 20 years from different countries and from different researchers clearly showed that the improvement of milk quality in the broader sense means also an increase of farmer revenues.

Even though the positive cost/benefit ratio itself is enough to support the efforts to reduce SCC, it must be emphasized that producing milk and dairy products of the highest quality and salubrity is a major moral duty for all the people involved in the dairy products chain (farmers, cheesemakers, veterinarians, agronomists, extensionists...).

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MILK QUALITY PAYMENT: QUALITY ASSURANCE (QA) IN SOMATIC CELL COUNTING

1 OBJECTIVE

To guarantee the uniformity and accuracy of somatic cell counting in all 18 official quality payment laboratories in Switzerland.

2 QA-ELEMENTS

- Basis:
  Technical prescriptions for laboratory testing of milk within the scope of the milk quality payment ("standard operating procedures")
- Monitoring within laboratories:
  a) "Pilot milk samples" (repeatability)
  b) Standard milk samples (bias)
  c) Comparative measurements
- Collaborative trials
- Maintenance of the cell count instruments
  - Routine maintenance according to the prescriptions of the manufacturer
  - Servicing of the instrument at least once a year by the manufacturer
- Training of the laboratory personnel

3 MONITORING WITHIN LABORATORIES

(a) "Pilot milk samples"

"Pilot milk samples" are used for routine monitoring of the repeatability of counts. For this purpose a raw milk sample with about 300 000–400 000 cells/ml (determined by fluoro-opto-electronic counting) is counted after every 20th sample throughout the working day. At the end of the day the coefficient of variation is calculated. If it is greater than 5% the results do not count for quality payment and the laboratory procedure has to be checked (especially the volumetrics and mixing).

(b) Standard milk samples ("milk standards")

In order to assess the counting bias within a routine laboratory, standard milk samples are used which are provided by the Somatic Cell Counting reference centre (FAM).

A sufficient amount of 2 "milk standards" with different cell count levels (approximately 25 litres/standard) is prepared by the reference laboratory every 6 weeks using whole milk (cell free, homogenized, UHT-treated milk), bovine blood leucocytes and preservatives. Standard "low" has a cell count of 150 000–200 000, standard "high" a cell count of 350 000–450 000 cells/ml.

The "true" count of each standard is estimated microscopically (IDF Standard 148A:1995) and additionally determined by fluoro-opto-electronic counting (50 consecutive measurements).

Every week the routine laboratories receive a set of 5 vials (approximately 40 ml/vial) of each "milk standard".

The "milk standards" have to be counted 5 times by each laboratory at the beginning of each series of analyses. If the mean count for either standard differs from its "true" count by more than 5% the calibration of the instrument or any other causes of systematic errors have to be checked. The instrument cannot be used for quality payment measurements until the causes of the error are eliminated and the standard measurements give satisfactory results. In most of the routine laboratories the instruments are computer-controlled and equipped with electronic data transfer to a PC. If the standard measurements differ from the "true" values by more than 5% the instrument is blocked automatically and routine counting is not possible.

Every week the results of the daily "milk standard" and pilot sample measurements are sent to the reference centre (written protocols or diskettes) where they are evaluated statistically for each laboratory and all laboratories together. The results of this analysis are sent back to the laboratories (Annex 1).

(c) Comparative measurements

One day every month each laboratory collects randomly 16–20 routine samples which were analysed for quality payment. The unpreserved milk samples are sent together with the respective cell count results to the reference centre where they are re-analysed.

The results of the two measurements are evaluated statistically and reported to the laboratories (Annex 2). If the results are not satisfactory the instrument has to be checked by the maintenance service of the manufacturer.

4 COLLABORATIVE TRIALS

The purpose of collaborative trials is to obtain estimates of the repeatability of counts of the same samples of milk in the 18 routine laboratories and to measure the bias in each laboratory's counts relative to the estimate of the "true" count of each sample.

Collaborative trials are carried out at least once a year. Design and statistical analyses are according to the IDF Standard 148A:1995 (Enumeration of Somatic Cells).

The reference laboratory participates itself regularly (4–5 times a year) in international collaborative trials (IDF, CECALAIT).

5 MAINTENANCE OF THE CELL COUNT INSTRUMENTS

Daily, weekly and monthly routine maintenance checks or repairs which are prescribed by the manufacturer have to be recorded.

Servicing of the instrument by the maintenance service of the manufacturer at least once a year is mandatory.

For calibration of the instruments (for example after major repairs or after the annual service) the reference laboratory provides microscopically counted, unpreserved raw milk samples.

6 PERSONNEL TRAINING

New laboratory personnel have to complete an introductory course organized by the reference laboratory and the manufacturer of the instrument.

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MASTITIS: THE DISEASE UNDER ASPECTS OF MILK QUALITY AND HYGIENE

(1) Inflammatory changes in the mammary gland influence the process of milk synthesis both quantitatively and qualitatively. The changes in the constituents of milk affect the major components (lactose, fat, proteins) as well as fatty acids, protein fractions, caseins, whey proteins, anions and cations, conductivity, enzymes, etc. With increasing numbers of somatic cells the growth of starter cultures in the milk may be adversely influenced. Renneting time and heat stability of the milk can be impaired.

(2) The hygienic value of raw milk is determined by pathogenic microorganisms, saprophytic microorganisms, residues and contaminants. In the case of mastitis pathogenic microorganisms may occur, which are infectious also for man (for example *B-streptococci, Escherichia coli* strains) or which, under certain circumstances, form toxins (for example *Staphylococcus aureus, Escherichia coli*).

(3) Residues due to the treatment of mastitis include primarily antimicrobials like antibiotics and sulfa drugs. The significance of antibiotic residues in milk has to be discussed under two importance aspects:
- that relating to payment of milk on the basis of the technological quality ("inhibitors") and
- that relating to public health and governed by food laws.

(4) Concerning the effect of antibiotic residues on human health, a number of aspects have to be considered (toxicological, microbiological and immunopathological effects). From a technological point of view, the sensitivity of thermophilic and mesophilic starters to different antibiotics may vary widely. The fixation of maximum residue limits (MRLs) for a number of antimicrobials has led to the difficulty that a negative result of so-called inhibitor tests does not necessarily indicate concentrations of antimicrobials below the MRLs. A new and integrated system for the detection of antimicrobials has to be developed, which takes technological and toxicological aspects into consideration.

(5) The use of disinfectants for the dipping of teats before and after milking must also be critically evaluated. Not only the active ingredients (iodine, chlorhexidine, chlorine etc.) have to be taken into consideration, but also other components used (for example detergents, additives).

(6) The number of somatic cells in milk is an indicator of its qualitative/hygienic properties, which can reflect the given mastitis situation in the herd. The objectives of mastitis control measures should primarily cover the supply of milk with a low somatic cell content, which meets the qualitative and hygienic expectations of manufacturers and consumers.

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NEW SYSTEMS FOR SOMATIC CELL COUNTS

For counting somatic cells in milk ("cell counting"), as part of the EC/EU- and national regulations, standardization is indispensable; this includes, in particular, the comparability of methods.

In the following results from our own studies on comparisons between the fluorescence optical system (Fossomatic 360, Foss Electric A/S, Hillerød, Denmark) three systems using flow cytometry are reported. The three systems are:
- Somascope (Delta Instruments b. v., Palweg 9, 9231 HW Surhuister veen, the Netherlands)
- Somacount (Bentley Instruments INC, Chaska, USA)
- ANADIS SCC 500 (ANADIS Instruments, 9, Rue de Pins d'Alep, 30100 Ales, France)

The results of the studies are published in detail for Somascope [1] and Somacount [2] or will be published soon (ANADIS).

In Figure 1 the comparisons of methods on quarter foremilk samples are given as scattergrams. The overall impression is, the three flow cytometric methods give similar results as compared with the fluorescence optical one in the range between approximately 50 000 and 1 000 000 cells/ml. Somascope tends to overestimate in the lower range and to underestimate in the higher range.

Somacount and ANADIS SCC 500 seem to differentiate cell counts below 50 000 better than Fossomatic 360.

In Figure 2 the results of intercomparisons with the different methods are given as scattergrams (maximum repeatability versus maximum bias). The "reliable portion" of participating laboratories is marked by a frame. Taking into account, that in intercomparison trials the applied method together with the respective proficiency of the laboratories are under study, one can state that, in comparison with the most recent Intercomparison No. 25 the results with the new systems are acceptable.

Figure 3 puts together the measures for repeatability (r) and reproducibility (R) expressed as coefficients of variation (cv_r and cv_R) computed from the results of the intercomparisons shown in Figure 2. For evaluation at a glance the lower tar-

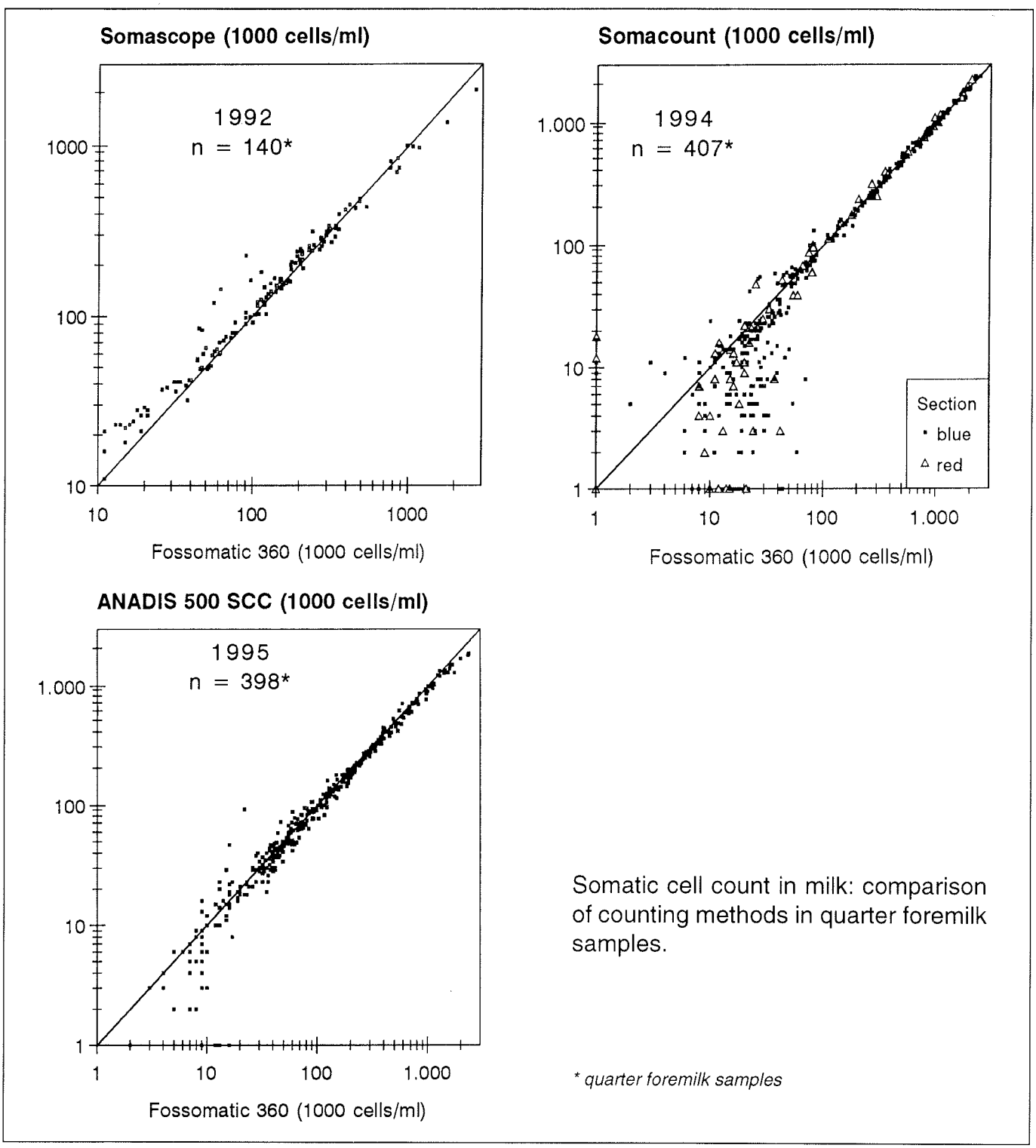

Figure 1

get values (IDF Standard 148A:1995) are given (cv_r: 10–5%; cv_R: 20–10% depending on the cell count level).

Besides the problems in the lower level of cell counts, which are still common to all systems, the impression from Figure 2 holds here too: all systems compared give acceptable results in general. Thus the choice of method can be based on practical aspects such as economics and service supply.

One remark at the end. The reliability of a cell count result as a parameter for the situation in the unit which was sampled depends mainly on the quality of the sample (taken representatively, handled properly). Even highly sophisticated methods can not compensate for mistakes at the root of the information line.

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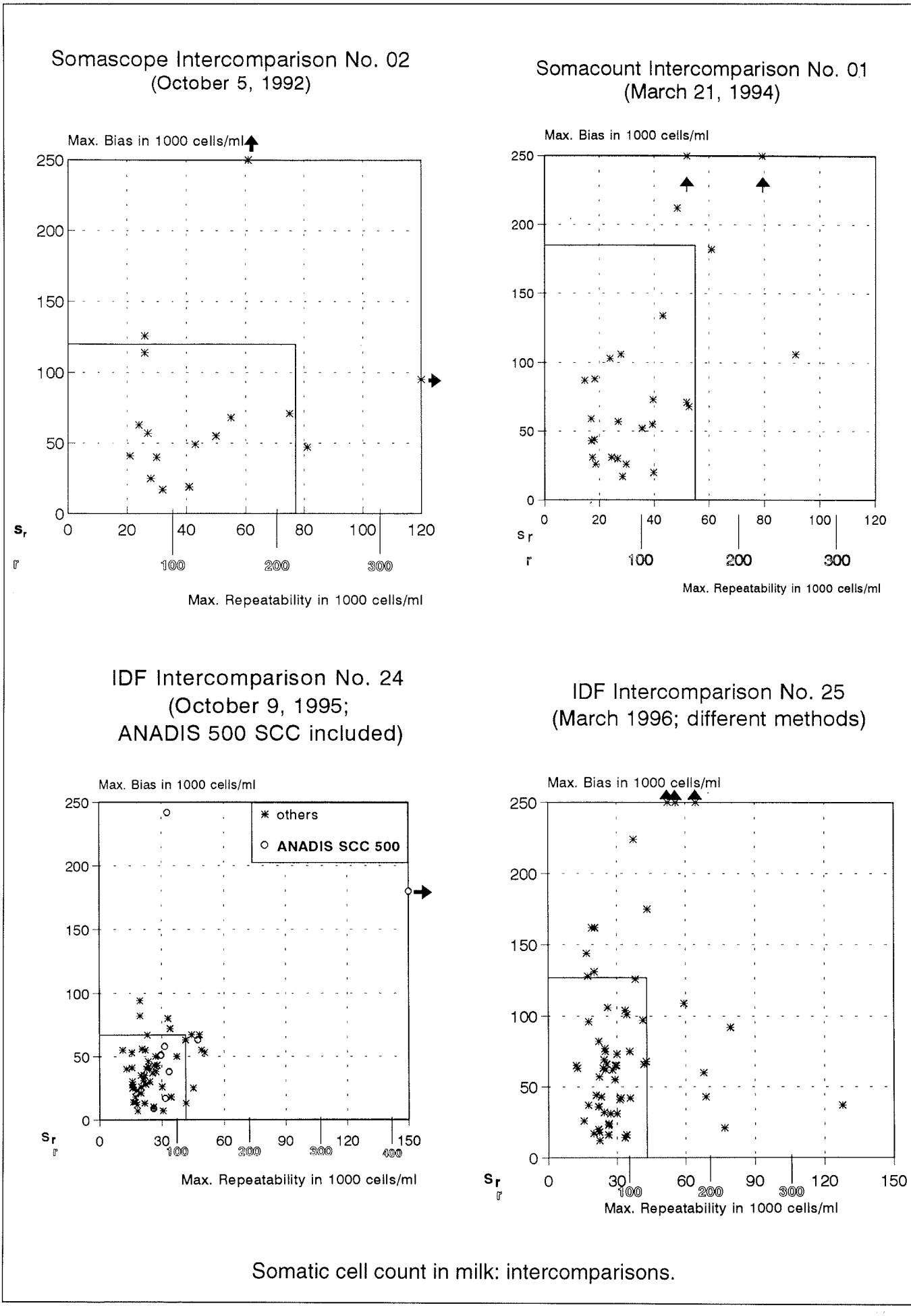

Somatic cell count in milk: intercomparisons.

Figure 2

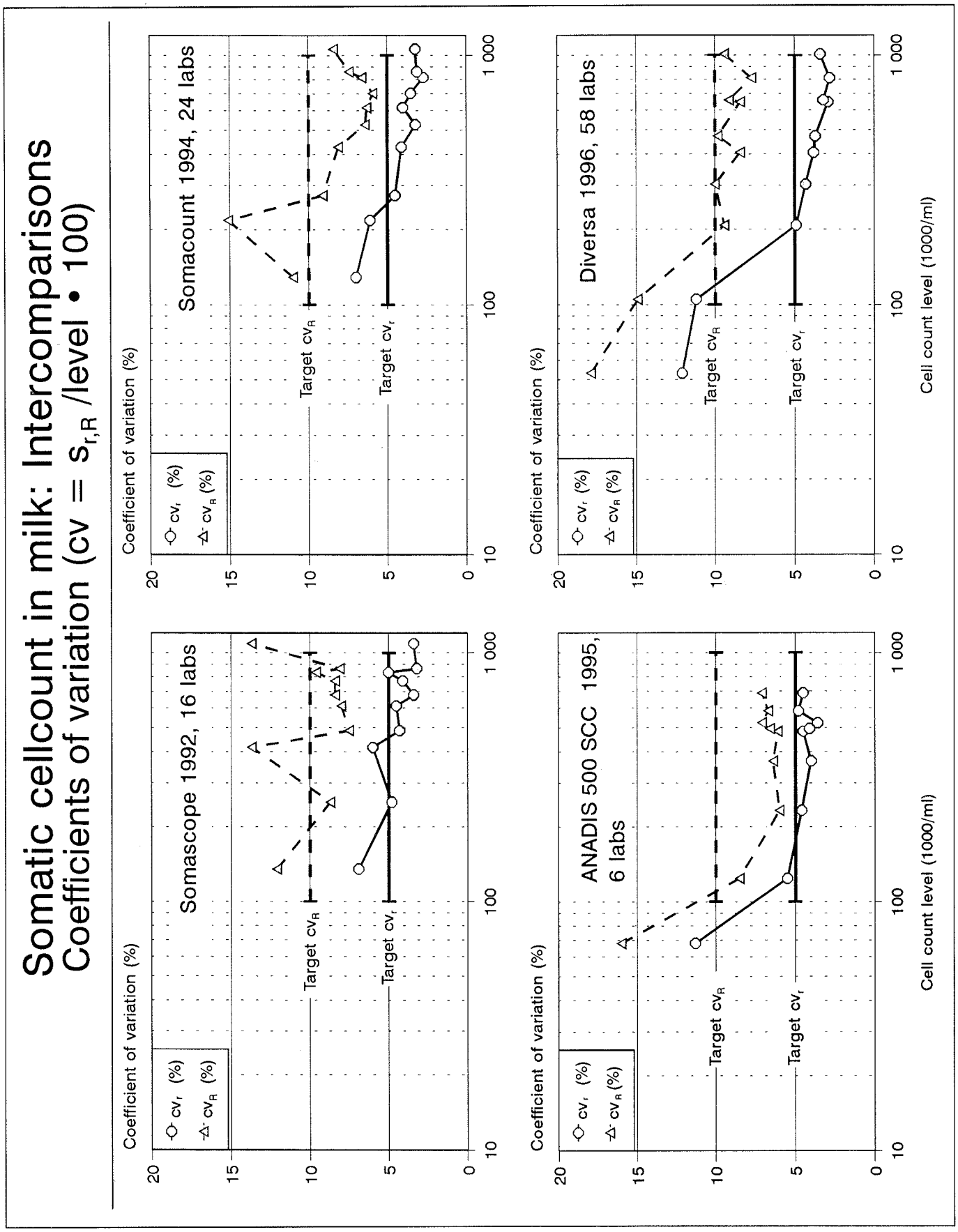

Figure 3

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Mastitis Notes from Member Countries

FINLAND

MASTITIS PREVENTION HAS SUCCEEDED IN FINLAND

In spite of Finland having a long tradition in bovine mastitis control work, the disease has accounted for substantial economical losses to farmers and the dairy industry during the past 50 years. The first formal mastitis control scheme was started in the 1940s to eradicate the contagious form caused by *Streptococcus agalactiae*. The official campaign was terminated in 1968 and from 1978 the mastitis control programme was continued on a voluntary basis by milk producers, the dairy industry, dairy advisers and veterinarians. Despite the control programme, the prevalence of mastitis increased during the 1970s and 1980s and consequently in 1988 another attempt by the Government of Finland to decrease mastitis prevalence and to increase the quality of raw milk was initiated.

An expert committee founded by the Ministry of Agriculture and Forestry proposed several means to decrease mastitis, including a decrease in the maximum acceptable bulk milk somatic cell count (SCC), a quality premium for milk, training of veterinarians, dairy advisers and milk producers in prevention of udder diseases, development of guidelines for diagnosis and treatment of mastitis, and preparation of teaching material. It was also suggested that the dairy industry should employ veterinarians specialized in production animals and increase the number of dairy production advisers helping the farmers in herd management.

These measures seemed to reduce mastitis prevalence. According to prevalence studies, 33% of cows had mastitis in 1975, 37% in 1980 and as many as 48% in 1988. A welcome change was noted in the 1995 study, in which the prevalence of mastitis was 38% (Figure 1). National bulk milk SCC data have been collected since 1988 and during the 1990s the mean SCC decreased to 132 000/ml (geometric mean) in 1995. In all the above-mentioned prevalence studies a cow was diagnosed as having mastitis if the CMT score in at least one quarter was 3 or higher, or the SCC was over 300 000/ml. The criteria for mastitis were quite strict in these surveys. If the IDF criteria with a threshold of 500 000 cells/ml were used, in the 1995 survey the prevalence of mastitis would have been 28%, not 38%.

The relative proportion of mastitis-causing microorganisms has changed during recent decades and general trends can be seen in Figure 2. Not all the factors affecting bacterial ecology of mastitis pathogens are clear but some conclusions can be drawn. *Str. agalactiae* mastitis began to decrease in the mid-1950s, now being close to zero. This change was due to two main factors: the end of the hand-milking era and the use of penicillin in therapy of *Str. agalactiae* mastitis. It also seems evident that the machine-milking was one impor-

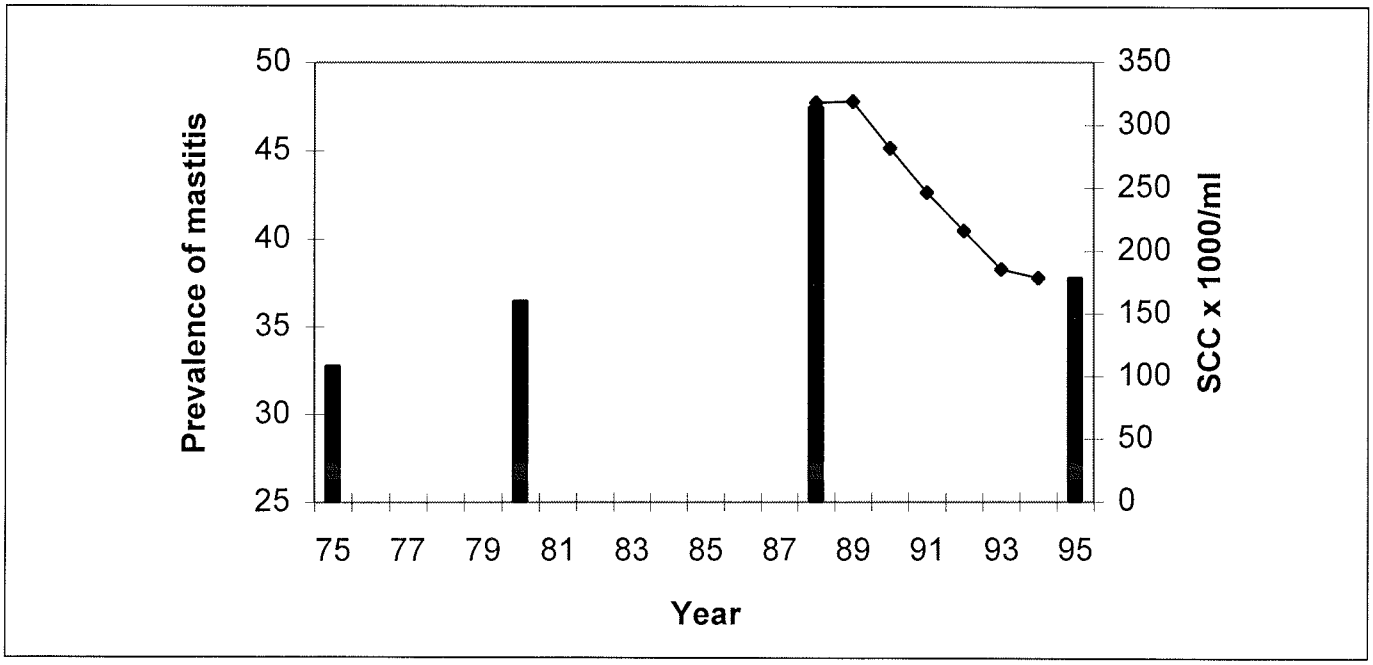

Figure 1: **Development of mastitis prevalence according to surveys (bars) and SCC of milk delivered to dairies (line) between 1988 and 1995 in Finland.**

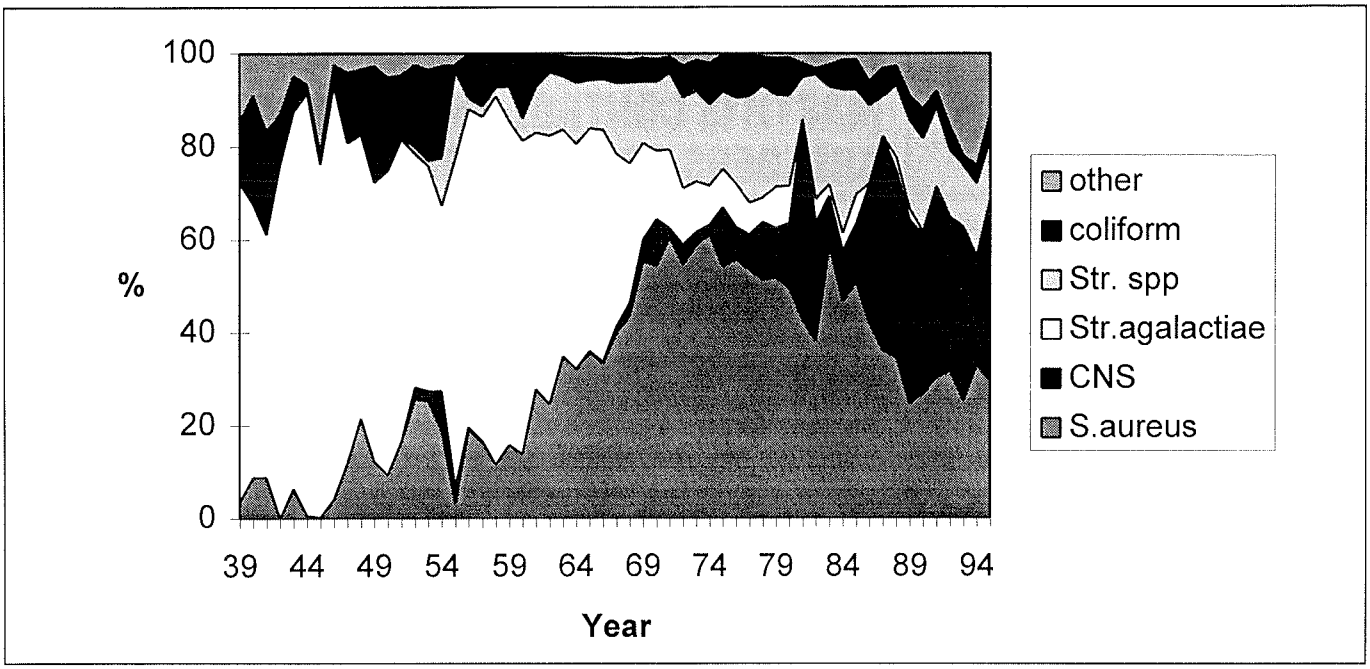

Figure 2: **Relative distribution of bacterial isolates recorded from quarter milk samples at the National Veterinary and Food Research Institute between 1939 and 1994.**

tant cause in the increase of *S. aureus* mastitis, and culling of chronic *S. aureus* cows was an effective means of decreasing *S. aureus* mastitis during the last years. As *S. aureus* is known to cause chronic subclinical mastitis, which increases SCC, it is likely that the decrease in mastitis prevalence in general, and decrease in the prevalence of *S. aureus* mastitis in particular, were correlated. It is not known why coagulase-negative staphylococci (CNS) are currently the most prevalent mastitis-causing bacteria, often producing quite mild clinical symptoms. Factors that may have affected the change in staphylococcal species could include intensive use of antibacterials. Intensive antibacterial treatments of clinical mastitis and dry cow therapy with broad spectrum antibacterials were commonly used during the 1980s in attempts to eradicate chronic *S. aureus* mastitis. Mastitis caused by Gram-negative bacteria has never been a major problem in Finland.

The susceptibility of staphylococci to antibacterials has also changed. The proportion of bacterial isolates resistant to at least one antibacterial drug increased among *S. aureus* isolates from 30.5% in 1988 to 63.6% in 1995, and in CNS from 26.6% to 49.7%. Most of the increase in antibacterial resistance was due to higher numbers of β-lactamase producers. Resistance to multiple antibacterials also increased, but the increase was proportional to the total increase in resistance (Table 1). CNS were more often resistant to several antibacterials than *S. aureus*. As the incidence of CNS mastitis seems to increase, the multiresistant CNS strains may become a serious clinical problem. They may also represent a reservoir of resistance genes to *S. aureus*. The mastitis streptococci have remained susceptible to all β-lactams.

Most of the measures suggested by the expert committee have been instituted and the work to decrease the prevalence of mastitis and increase the quality of raw milk has succeeded very well. This has been possible because of the work of well motivated farmers, but the maintenance of this situation still represents a substantial task. In the future it is probably going to be more difficult to manage dairy farming in Finland profitably and this may mean less attention is devoted to preventive mastitis work. Moreover, the role of antimicrobial therapy is being re-evaluated. The development of bacterial resistance has to be followed systematically to avoid problems caused by multiresistant mastitis microbes in the future.

Table 1: Proportion of susceptible staphylococcal strains in antimicrobial susceptibility testings (1988 and 1995)

	S. aureus		CNS	
	1988 n = 344	1995 n = 154	1988 n = 237	1995 n = 183
Cephalotin	99.7	98.0	99.1	97.3
Erythromycin	95.9	97.4	90.7	88.5
Enrofloxacin	99.7	100.0	99.1	98.9
Neomycin	98.8	96.7	97.5	97.3
Oxacillin	98.5	100.0	98.7	99.4
Penicillin	68.2	49.3	75.9	62.8
Tetracyclines	93.0	88.3	89.9	90.2
Trimethoprim-sulfa	99.4	100.0	100.0	91.7

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ITALY

MASTITIS CONTROL PROGRAMME AND BREEDERS ASSOCIATION

Modena province is a relatively small area in the northern part of Italy. The dairy industry has an important role in the economy of the province. A large part of the milk produced is devoted to the production of one of the best known Italian cheeses: Parmigiano Reggiano (Parmesan).

The number of cows in the province was 72 617 in 1992; 25.7% of them were under the control of the local breeders association (APA). A large part of the cows are Italian Holstein (Frisona Italiana), but some local breeds are still used, particularly on small hilly farms.

Parmigiano Reggiano is a hard cheese which must be aged for at least 2 years. Its production is affected largely by the quality of milk, both in terms of cheese yield and quality.

The presence of mastitis (clinical or subclinical), increasing somatic cell counts, affects cheesemaking processes because:

- it reduces cheese yield efficiency
- it affects the aging processes
- it increases the number of defective (poor quality) cheese pieces.

The relationship between SCC and milk yield is well known; therefore, farmer losses due to SCC for cows under the control of the APA can be estimated (Table 1).

The overall losses due to reduced milk yield can be estimated as 3800 million Lit, which means, at the 1992 currency rate, 3 000 000 $ losses.

Table 1: Milk yield losses/year (prices in Lit.)

Linear Score	SCC (x1000)	Cows	%	Losses/cow kg milk	Losses/cow Lit (x1000)	Total losses kg milk	Total losses Lit (x1000)
0	0–17	398	2.1				
1	18–34	1103	5.9				
2	35–70	2607	13.9				
3	71–140	3613	19.3	167	100.2	603 371	362 023
4	141–282	3931	21.0	334	200.4	1 312 954	787 772
5	283–565	3508	18.8	501	300.6	1 757 508	1 054 505
6	566–1130	2287	12.2	668	400.8	1 527 716	916 630
7	1131–2262	938	5.0	835	501.0	783 230	469 938
8	2263–4525	269	1.4	1002	601.2	269 538	161 723
9	>4526	45	0.2	1002	601.2	45 090	27 054
				TOTAL		**6 299 407**	**3 799 644**

Milk price Lit 600 (≅ 0.4 $) 1992.

Table 2: Cheese yield losses/year (prices in Lit.)

SCC (x1000)	Cows	Casein on total protein	Milk casein %	Casein yield/cow 100 kg
0–282	11 652	0.77	2.40	1.51
283–565	3508	0.75	2.34	1.47
566–1130	2287	0.71	2.22	1.39
>1130	1252	0.69	2.15	1.35
TOTAL	**18 699**			

SCC (x1000)	Cows	Cheese yield/ cow 100 kg	Cheese yield/ cow (Lit x1000)	Total cheese yield 100 kg	Total cheese yield (Lit x10^6)
0–282	11 652	4.07	4880	47 389	56 866
283–565	3508	3.96	4754	13 896	16 676
566–1130	2287	3.75	4500	8576	10 292
>1130	1252	3.84	4373	4563	5 475
TOTAL	**18 699**			**74 424**	**89 310**

1992 cheese prices 12 000 Lit/kg (≅ 10 $).

The relationship between SCC and milk composition is well know, therefore we can estimate also the losses regarding the cheesemaking process (Table 2).

The data estimate very well what benefits could be gained from an improvement of mammary gland health status (a reduction of SCC) in the area.

On these bases, in 1993 the APA decided to build a mastitis diagnostic laboratory. The laboratory has been organized with the help of the Institute of Infectious Diseases (Mammary Pathology Center) in Milan and till now represents a unique experience in our country. This laboratory completed the number of services already offered to the breeders: individual SCC (monthly), machine milking control and maintenance, breeding and veterinary assistance.

After the first year spent tuning up the different persons involved (technicians, practitioners and farmers), an udder health monitoring programme has been started. This project involves 6 practitioners, 10 technicians (4 in the laboratory, 6 in the field) and is financed by the farmers, who pay a fee for each service, and by regional funds.

Up to now more than 100 farmers have applied for the project, mainly with the aim to reduce SCC. The results showed that the major problems are due to *Staph. aureus* and environmental mastitis. The joint efforts to improve management, control milking machine by the field technicians, a regular and precise monitoring of udder health by the laboratory and a more efficient use of therapeutics have produced positive results in a large number of the farms.

At the end of this year the statistics will be available on the work done, but the increasing number of farmers asking for APA services are strong signals that the project is reaching its target.

This experience confirms, if needed, that a strong cooperation between field, breeders association and research is the only way to promote a consistent and continuous progress in herd health programmes.

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NORWAY

BULK MILK SOMATIC CELL COUNT IN GOAT MILK
(A PRESENTATION ACCORDING TO NEW STANDARD)

IDF has completed a work making recommendations when presenting somatic cell count results. This paper demonstrates the principles of the recommendations on a material of bulk milk somatic cell count (BMSCC) in goat milk in Norway (1993, 1994 and 1995). The goat BMSCC results are also compared with the results of cow milk in Norway 1995.

The IDF makes the following recommendations regarding presentation of SOMATIC CELL COUNT (SCC) data:

Calculation of **means and distributions:**

- Geometric mean or mean of natural logarithm (ln or e) of SCC with standard deviation (std)
- Mean and confidence interval of logarithmic transformed SCC-data converted back to natural figures
- Percentage of data below fixed figures based on appropriate decimal deviation (20, 30, 40, ..., 100, 200, 300..., 1000, 2000, 3000 etc.)
- The limit for data within 10%, 20%, 30% etc. of the figures (Percentiles)

For calculation of **SCC in a batch of composite milk:**

- Weighted (by milk yield for the unit analysed) arithmetic means

SAMPLING IN NORWAY

In Norway the milk is delivered to the dairy every second or third day. Every herd is sampled twice a month for analysis of the bulk milk according to protein, fat, lactose and somatic cell counts. The equipment used is Fossomatic 360, Hilleröd, Denmark. In this material no sample is excluded. One problem with goat milk is the skew distribution of the kidding season. This seems to affect the SCC level during the year as shown in Figure 1, which presents the geometric mean during 12 months and distribution of the single sample results.

DISTRIBUTION OF HERDS ANNUAL GEOMETRIC MEAN

The herds' annual geometric BMSCC mean is calculated as a horizontal calculated mean of all results generated from each herd (within unit at different time). This annual geometric mean is, from 1 January 1995, the basis for quality payment according to BMSCC for goat milk in Norway.

The distributions for the herds' geometric mean BMSCC during the years 1993 to 1995, as well as correspondent figures from cow milk in Norway during 1995, are presented in Tables 1–3.

The distribution of decimal range is stopped at the figure having less than 2.5% and more than 97.5% of the data (Table 3).

The limits for premium classes for BMSCC in goat milk are set at <1200, 1200 to 1500 and no premium > 1500 ml^{-1}. In 1995, the first year of quality payment for goat BMSCC in Norway, 82.2% of the herds received 4% increase in payment (< 1200), 12.8% received 2% increase (1200 to 1500) and 5% did not receive any increase in payment (> 1500). The farmers would adapt to the new quality payment for BMSCC in goat milk. This is a very first step to heighten the quality of goat milk, which is really needed.

The goat milk is also paid according to the total content of fat, protein and lactose since January 1995. Lactose is also correlated to udder health.

Table 1: Distribution of Norwegian goat herds' annual geometric mean BMSCCs (1000 SCC ml^{-1}) for the years 1993–1995.Corresponding geometric mean of the cow herds samples during 1995

Variable	Variable for species and year			
Species	goat			cow
Year	1993	1994	1995	1995
Number of herds	880	904	861	25 625
Mean ln of BMSCC	6.80	6.85	6.81	4.82
std of ln BMSCC	0.36	0.32	0.32	0.47
Lower confidence interval	433	496	478	4
Exponential of ln BMSCC (geometric mean)	897	945	907	124
Upper confidence interval	1854	1803	1560	305

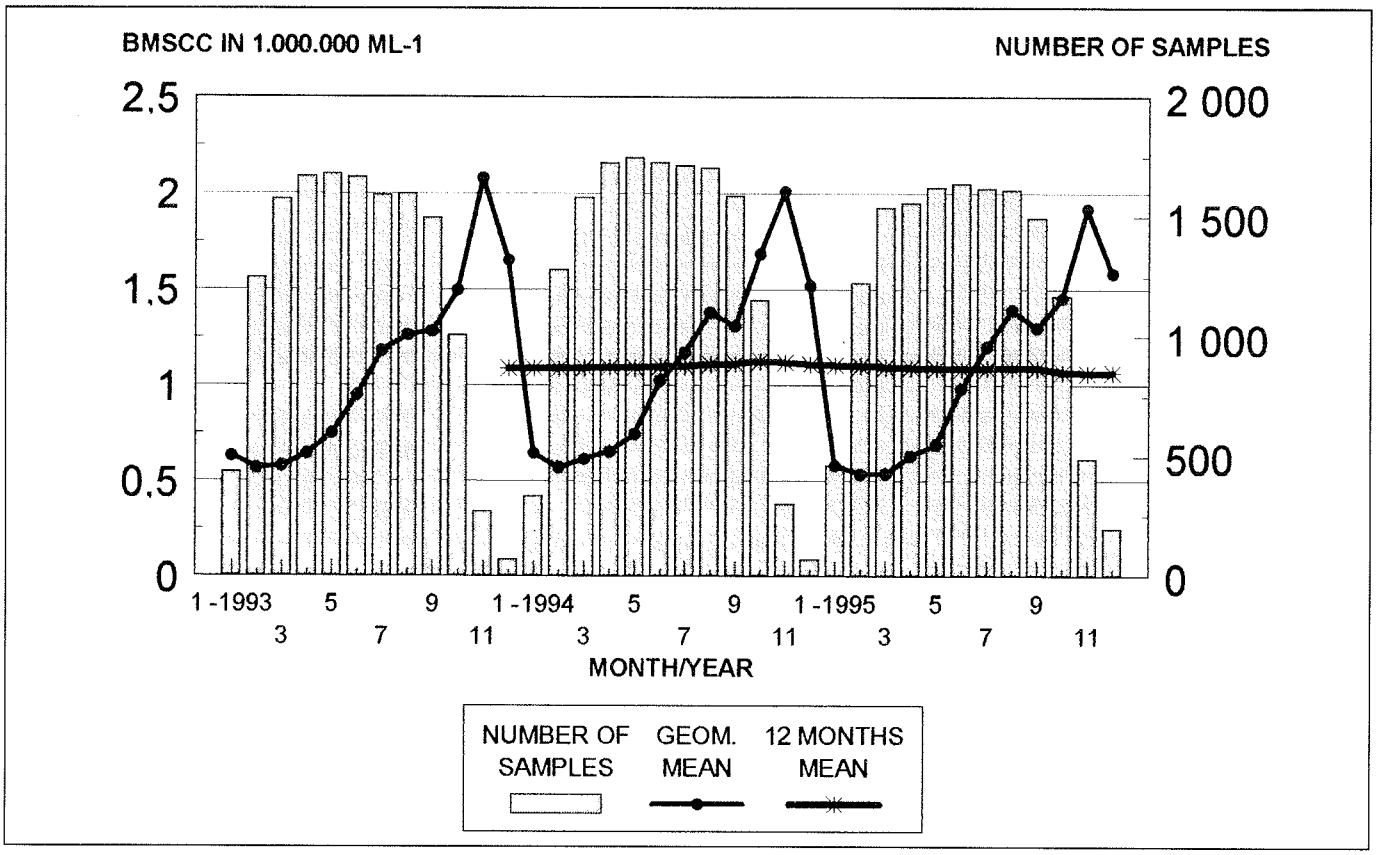

Figure 1: **Number of samples analysed for somatic cell counts from bulk milk (BMSCC) in goat herds in Norway 1993, 1994 and 1995. The monthly geometric mean, as well as the rolling 12-month period geometric mean, are presented.**

Table 2: Upper limit values (BMSCCs in 1000 ml^{-1}) for each percentile for the years 1992, 1993, and 1994 in goat milk annual geometric mean. Distribution of cow herds' annual geometric means in 1995

Percentiles	BMSCC in 1000 ml^{-1}			
	goat			cow
	1993	1994	1995	1995
10	540	620	610	67
20	660	720	696	85
30	760	800	771	100
40	840	880	836	114
50	920	950	906	128
60	1010	1030	980	143
70	1100	1130	1074	161
80	1200	1230	1173	184
90	1390	1410	1349	219
Number of herds	880	904	861	25 625

Table 4: Distribution of single results from BMSCC in Norwegian goat milk. Geometric mean BMSCCs (1000 SCC ml^{-1}) for the years 1993 to 1995

Variable	Value each year		
	1993	1994	1995
Number of samples	14 308	14 926	14 631
Mean ln of BMSCC	6.80	6.85	6.82
std of ln BMSCC	0.65	0.63	0.65
Lower confidence interval	245	268	248
Exponential of ln BMSCC (geometric mean)	897	943	916
Upper confidence interval	3313	3352	3368

Table 3: Cumulative frequency distribution of goat herds according to their annual geometric mean BMSCC (1000 SCC ml^{-1}) 1993 to 1995. Distribution of the cow herds annual geometric means during the year 1995

BMSCC	goat			cow
upper range	1993	1994	1995	1995
40				1.7
50				3.9
60				7.3
70				11.6
80				17.1
90				23.3
100				30.3
200				85.3
300				98.1
400	1.8	0.7	0.9	
500	5.9	2.5	3.5	
600	13.5	8.1	8.9	
700	24.8	18.5	20.4	
800	34.4	29.4	34.6	
900	47.3	42.7	49.5	
1000	59.1	55.9	62.4	
2000	98.5	99.0	99.4	
Number of herds	880	904	861	25 621

DISTRIBUTION OF SINGLE SAMPLE RESULTS

The distributions of the single sample results are different. Such a presentation is named vertical calculation (within time at different units). Corresponding figures to Tables 1–3 from distribution of single sample results are presented in Tables 4–6.

Compared to the distribution of each herd's geometric mean (Table 1) the mean ln of BMSCC would be the same figure, but the distribution expressed as std and upper and lower confidence interval would be different. One should be aware of per se differences in presenting figures from primarily horizontal calculation (for example for herds or animal during a year) and a simple vertical calculation using each single observation. The same problem would be presented in Table 5 and 6 comparing Table 2 and 3.

Table 5 illustrates that the median (50% percentile) would be nearly the same as in Table 2 (distribution of herds' geometric means during a year), but the percentiles at lower and higher level would be lower and higher, respectively. Table 6 presents the percentage of samples taking a fixed number or lower numbers of BMSCC.

SEASONAL DISTRIBUTION

As Figure 1 shows, one of the major problems in analysing for goat BMSCC is the skew distribution of samples reflecting the concentrated kidding season during winter time. The geometric mean BMSCC is fairly low during the first indoor season from February to May, then elevated during summer, and even higher in the autumn.

QUALITY PAYMENT ACCORDING TO BMSCC IN GOAT MILK

The problem of the skew distribution presents huge difficulties in establishing a quality payment scheme. In Norway this was solved by calculating a 12-month period geometric mean for 1995. Premium quality is given for herds with the lowest BMSCC in relation to that figure. From next year (1997) the quality premium will be paid each month according to the previous 12-month period rolling geometric mean.

Quality payment according to BMSCC in goat milk has never been done before in Norway, neither are we aware of other countries having a premium quality for goat milk according to BMSCC. In Norway the figures are fairly low for cow milk (Tables 1–3). This good result is partly due to a quality payment scheme according to BMSCC running for over 10 years now, and partly to the advisory work done through the national udder health programme started in 1982. In goat milk, figures of 540 000 ml^{-1} in hand-milked herds and a level of 720 000 ml^{-1} in machine-milked herds have been observed [1]. These figures comparing the results presented in this article indicate that the machines and milking management have not made any progress in relation to udder health during the last 20 years. Results from Østerås & Brenne [2] indicated a non-linear correlation of BMSCC in goat milk and protein content in milk, with higher protein concentration at BMSCC above 900 000 ml^{-1} and below 600 000 ml^{-1}. This could indicate two components in the protein fraction: one of casein (decreasing according to BMSCC) and another of immune or blood origin (increasing with increasing BMSCC). If breeding and quality payment of goat milk is done only according to protein content, this could cause a selection for milk high in BMSCC which correlates to higher protein content. Such proteins are not supposed to be suitable for good milk products, presenting problems both with organoleptic quality and cheese manufacture. This problem could be solved by correcting for the effect of protein by introducing udder health criteria as BMSCC or lactose in breeding indexes and quality payment schemes. The experiment with 12-month period geometric mean and lactose done in Norway during 1995 is the first step in such a correction. Further steps will be taken when farmers have adapted to the new system and proper advisory schemes are in place. The goal for goat BMSCC should, according to results in literature, be at levels around 500 000 ml^{-1}, perhaps even lower.

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Table 5: Upper limit values (BMSCCs in 1000 ml^{-1}) for each percentile for the years 1992, 1993, and 1994. Distribution of single sample results from bulk milk samples in goat herds

Percentiles	BMSCC in 1000 ml^{-1} each year		
	1993	1994	1995
10	400	420	400
20	530	560	530
30	650	680	660
40	770	810	780
50	910	950	930
60	1060	1110	1080
70	1260	1330	1290
80	1550	1620	1580
90	2010	2090	2050
Number of samples	14 308	14 926	14 631

Table 6: Cumulative frequency distribution of sample results according to their BMSCC (1000 SCC ml^{-1}). Distribution of the single sample results in all goat herds in Norway during the years 1993 to 1995

BMSCC upper range	year		
	1993	1994	1995
<200	1.6	0.9	1.3
300	4.5	3.4	4.5
400	10.1	8.6	10.4
500	17.6	15.8	18.0
600	26.2	23.9	25.9
700	34.4	31.8	33.9
800	42.6	39.7	41.5
900	49.8	46.7	48.4
1000	56.3	53.8	55.1
2000	89.8	88.5	89.4
3000	97.2	96.9	96.9
4000	98.9	98.8	98.9
Number of samples	14 308	14 926	14 631

SWEDEN, NORWAY, DENMARK & FINLAND

ANTIMICROBIAL DRUG POLICY IN FOUR NORDIC COUNTRIES

The dairy industry in the Nordic countries has a joint association called NMSM. One of the working groups within this association deals with health aspects in the dairy cattle population in the Nordic countries. A small investigation has been carried out on the principles for use of antimicrobial drugs in the dairy cattle population. Four of the five countries have been included in the study: Denmark, Norway, Finland and Sweden.

The structure of the dairy farming as regards number of dairy herds and herd sizes is fairly uniform in Denmark and Sweden, Norway and Finland, respectively (see Figures 1 and 2).

BREEDS/MILK YIELD

In Denmark and Sweden a large proportion of the dairy cattle population consists of the American Holstein breed together with the domestic red breeds. In Norway and Finland the domestic red breeds (NRF, Ayrshire) are the dominating breeds. The average milk yield from the four dairy cattle populations is presented in Figure 3.

ANTIMICROBIAL DRUG POLICY IN MASTITIS THERAPY

Clinical mastitis

The structure of the dairy cattle health service and the large animal practitioners' organizations are slightly different in the four countries. Still, the principles for the use of anitmicrobial drugs for treatment of mastitis are fairly similar (see Table 1).

In all four countries, a veterinarian always initiates the treatment of a cow with clinical mastitis. As a general principle, the mastitis therapy is commenced with an intramuscular injection of the drug. In Sweden the farmer is then allowed to complete the therapy with i.m. injections during the following 3–4 days. In 70–75% of the cases, the veterinary practitioner also takes a milk sample for bacteriological analysis in his own laboratory. The therapy is generally started with the use of penicillin. The choice of drug can then be changed on the second day if Gram-negative bacteria are isolated on the culture plates. In the other three countries, the initial intramuscular injection is usually followed by intramammary treatment for another

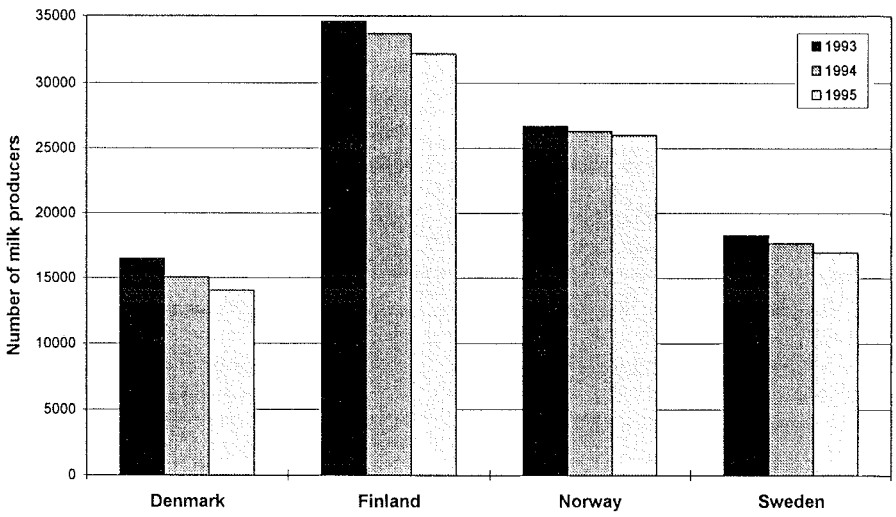

Figure 1: **Number of milk producers in the Nordic countries.**

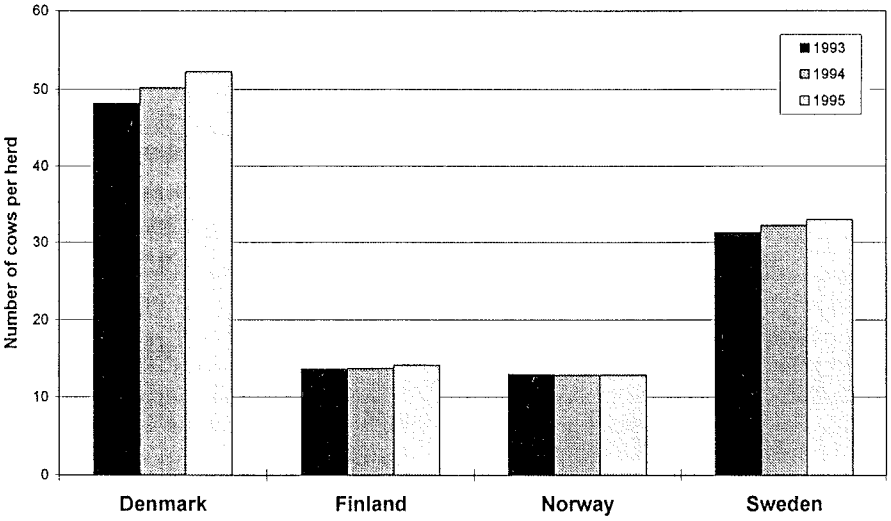

Figure 2: **Average number of cows in recorded herds.**

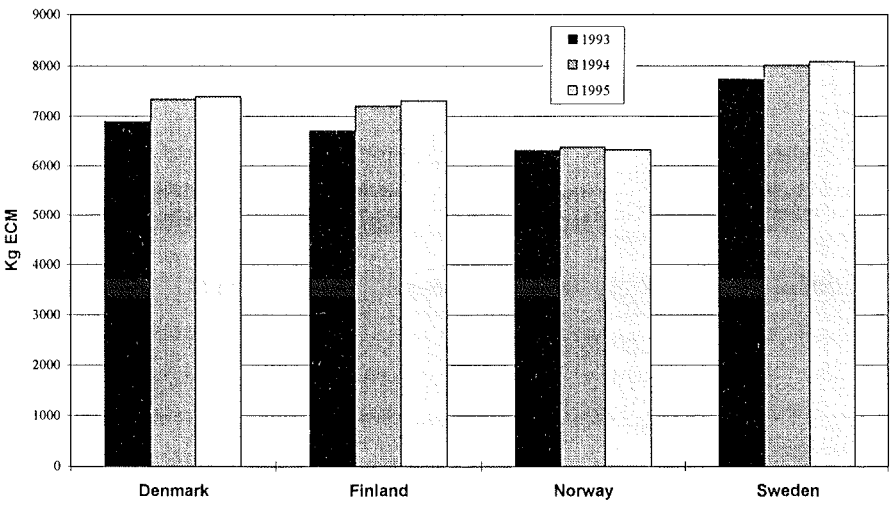

Figure 3: **Average milk yield, kg ECM.**

1–4 days, carried out by the farmer himself. However, in Denmark the farmer is not allowed to do this on his own unless he has joined a dairy cattle health service programme with monthly veterinary visits on the farm.

The farmer can not get access to drugs in any way other than from his veterinarian, that is, the vets have full responsibility for the principles of drug use in the dairy cattle population. Every treated case has to be recorded by the vet; still there are inconsistencies in the registration principles for the drugs used, which makes the control inefficient. Different kinds of compulsory recording routines are now being developed in these countries.

Dry cow therapy

Dry cow therapy is commonly carried out by the farmer himself by means of intramammary drugs. In the few cases where the dry cow therapy is initiated with an intramuscular injection, this is done by a veterinarian. In Denmark, by law, dry cow therapy is only allowed if milk sampling for bacteriological analysis precedes the treatment and after confirmation of mastitis pathogens, or if a veterinarian has identified clinical signs of mastitis. No more than the infected quarter is then allowed to be treated with antibiotics. In Norway, only short-acting lactation formulae are used in dry cow therapy.

Antimicrobial drug resistance

During the last year and a half, alarm reports have come from many parts of the world on growing problems with antibiotic-resistant bacterial strains, mainly from the human population. In the Nordic countries there is however no clear evidence of increasing problems with antibiotic-resistant strains in the dairy cattle population. Still, strong efforts are now being made to restrict the use of antibiotics in dairy cattle practice in order to avoid the development of resistant bacterial strains.

All the countries have formulated an antimicrobial drug policy with the common goal to reduce to a minimum the general use of antibiotics in the dairy cattle practice. As an example, the goal in Norway is to reduce the general use of antibiotics by 25% within 5 years. In Sweden, the dairies are also trying different ways to support the farmers in minimizing drug use, for example by quality payment systems. The dairy cattle health group is now making efforts to get a consensus decision on a future common antimicrobial drug policy for the 4 Nordic countries.

Table 1: Treatment by a veterinarian/farmer

Nordic country	Systemic		Intramammary		Dry cow intram.	
	Vet	Farmer	Vet	Farmer	Vet	Farmer
Denmark	+ initial	(+)[1]	+ initial	(+)[1]		+[2]
	+		+		+	
Finland	+ initial	+	+	+	+	+[3]
Norway	+ initial	+	+	+	+	+
Sweden	+ initial	+	+	+	+	+

[1] *For 5 days, provided that the farmer has an animal health care agreement with the veterinarian in accordance with the rules laid down by the State Veterinary Service.*

[2] *Provided that pathogenic microorganisms are diagnosed in quarter milk samples within 35 days, the laboratory diagnosis should include test of bacterial resistance.*

[3] *After laboratory diagnosis.*

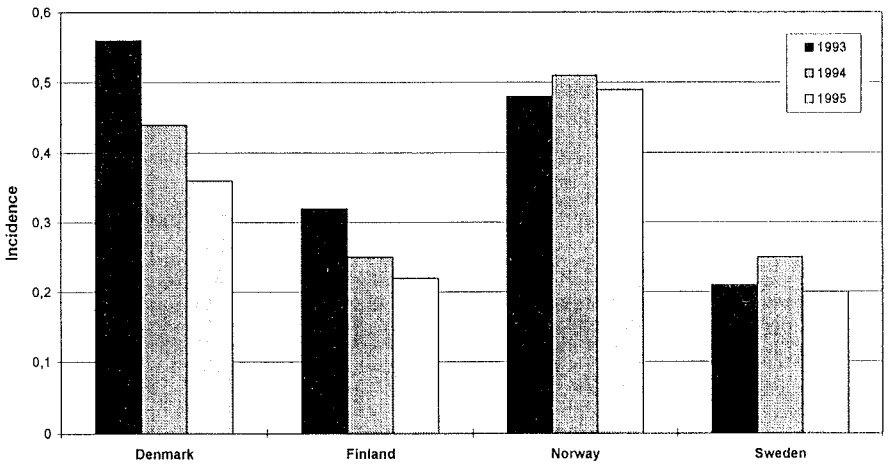

Figure 4: **Incidences of clinical mastitis in the Nordic countries.**

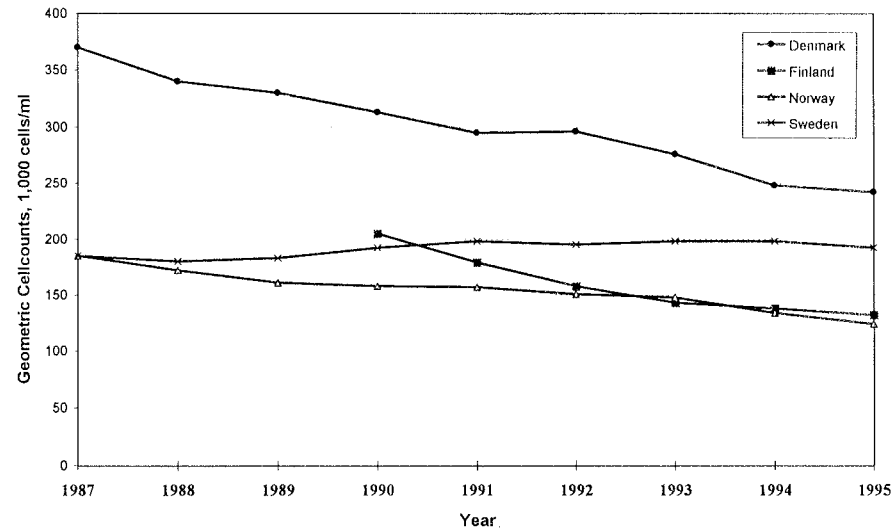

Figure 5: **Geometric mean cell counts in bulk milk.**

MASTITIS INCIDENCE/CELL-COUNT LEVELS

Over the last 3 years the group has made calculations on the incidence of clinical mastitis as well as geometric cell count levels in bulk milk, which are presented in Figures 4 and 5.

The figures should be evaluated cautiously, as the registration systems in the four countries are far from identical, making an exact comparison impossible. In Norway, for example, the treatments made by the farmers are included in the statistics, which is not the case in the other three countries.

The development of the cell count levels also shows an interesting tendency, with a continuous decline of the cell count levels in three of the four countries, whereas the Swedish cell count level remains constant.

The veterinarians from the four countries now continue their discussions in order to evaluate the reasons why differences actually exist between the countries. Among other things, the total amount of antibiotics used in relation to different principles for mastitis therapy are evaluated.

SUMMARY

The general principles for mastitis therapy in the four Nordic countries are as follows:

- The initiation of mastitis therapy is done by a veterinarian.
- The dairy farmer is allowed to make the systemic or intramammary treatments to complete the therapy.
- The therapy is selective, that is, broad-spectrum antibiotics are avoided.
- The countries are setting up antimicrobial drug policies to reduce the amount of antibiotics in order to avoid future problems with resistant bacterial strains.
- The veterinary group will now continue with the efforts to analyse the difference between the principles for mastitis therapy and their consequences in the four Nordic countries.

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IRELAND

ANTIBIOTIC RESISTANCE TESTING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CASES OF BOVINE MASTITIS IN IRELAND

An investigation was carried out to study antibiotic resistance in *Staphylococcus aureus* isolated from cases of bovine mastitis. One hundred and eighty-two isolates of *S. aureus* were collected from subclinical and clinical cases of mastitis in the South, East and North of Ireland. The isolates were tested against the following range of antibiotics : penicillin G, cloxacillin, cephalothin, erythromycin, novobiocin, neomycin and tetracycline. The antibiotic disc assay method was used (modified Kirby-Bauer). Culture concentrations equivalent to 0.5 McFarland standard were used after 4 h incubation at 37°C. The results are presented in Table 1.

Two culture incubation times (4 h and 18 h) were also compared in a subset of 119 of the 182 isolates. In this study, three additional antibiotics – framycetin, gentamycin and kanamycin – were also tested. The diameter of the zone of inhibition was reduced for all of the antibiotics on the 18 h cultures when compared to the zones on the 4 h cultures. The numbers and percentages of isolates that were resistant to the antibiotics at each of the incubation times are presented in Table 2.

The results showed that with 4 h incubation, 66% of the *S. aureus* isolates were resistant to penicillin G, and there was no resistance to either cephalothin or cloxacillin. There was very little resistance to the other antibiotics. The 18 h culture incubation times did reduce the diameter of the zones of inhibition for all antibiotics and altered the sensitivity of some. The results highlight the importance of following recommended standard procedures for antibiotic resistance testing which presently advocate 4 h incubations for test cultures.

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Table 1: Antibiotic resistance in 182 isolates of *S. aureus* from cases of bovine mastitis in Irish dairy herds

Antibiotic	Concentration/disc	Resistant (n)	Resistant (%)
Penicillin G	10 iu	120	66
Cloxacillin	5 mcg	0	0
Cephalothin	30 mcg	0	0
Erythromycin	15 mcg	1	0.5
Novobiocin	30 mcg	3	1.6
Neomycin	30 mcg	1	0.5
Tetracycline	30 mcg	7	3.8

Table 2: Antibiotic resistance patterns in 119 isolates of *S. aureus* in a comparison of 4 h and 18 h culture incubation times

Antibiotic	Concentration/disc	Resistant at 4 h (n)	(%)	Resistant at 18h n	(%)
Penicillin G	10 iu	73	(61.0)	77	(65)
Cloxacillin	5 mcg	0		0	
Cephalothin	30 mcg	0		0	
Erythromycin	15 mcg	1	(0.8)	20	(17)
Novobiocin	30 mcg	0		10	(8)
Neomycin	30 mcg	0		7	(6)
Tetracycline	30 mcg	4	(3.4)	34	(29)
Framycetin	100 mcg	0		0	
Gentamycin	10 mcg	0		45	(38)
Kanamycin	30 mcg	0		82	(69)

IRELAND & USA

ANALYSIS OF DIVERSITY OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM BOVINE MASTITIS USING DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF rRNA GENES

Analysis of DNA restriction fragment length polymorphisms of rRNA genes (ribotyping) was employed to study the diversity of *Staphylococcus aureus* isolates causing bovine mastitis in Ireland and in the USA. This method has been used extensively to study the population genetics and epidemiology of a wide range of bacterial species including *S. aureus*.

Altogether, 71 isolates were studied; 42 from the USA and 29 from Ireland. In all, 14 different ribotypes were represented amongst the 71 isolates. All of these were present amongst the American isolates, whereas only 4 different ribotypes were found amongst the Irish isolates.

Different ribotypes were produced by strains in the same herd, suggesting heterogeneity of strains within herds. Only one ribotype was produced by isolates from any single cow, suggesting homogeneity of isolates within individual animals.

There did not appear to be a correlation between different forms of the disease (that is, subclinical/clinical) and ribotyping.

Overall, the relatively few ribotypes found amongst the isolates studied suggests a largely clonal nature of *S. aureus* causing bovine mastitis, with only a few genotypic clonal types being isolated from animals with the disease. This knowledge should be of importance, as a rational and effective strategy for control of intramammary infection may need to be directed against clones that commonly cause disease.

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SWITZERLAND

MASTITIS PATHOGENS IN SWITZERLAND 1988–1994

Samples taken by Veterinarians from Cows with Clinical Mastitis

Results	1988 N=80 604 %	1989 N=83 520 %	1990 N=96 958 %	1991 N=80 078 %	1992 N=98 293 %	1993 N=83 330 %	1994 N=99 549 %
Bact. negative	**22.3**	**17.0**	**22.2**	**17.2**	**15.7**	**14.0**	**13.3**
Bact. positive (= 100%)	**77.7**	**83.0**	**77.8**	**82.8**	**84.3**	**86.0**	**86.7**
Streptococcus agalactiae	2.9	2.8	2.8	2.6	2.2	1.9	1.6
"Other streptococci" (*S. uberis*, *S. dysgalactiae*, enterococci)	32.0	31.5	32.9	32.4	36.6	35.6	36.1
Staphylococcus aureus	30.9	31.4	26.4	25.1	28.5	29.1	27.0
"Other staphylococci" (*S. epidermidis*,*S. xylosus*, *S. hyicus*,Micrococcus sp.)	11.4	12.9	17.5	17.9	14.8	13.3	14.0
Coliforms	12.7	10.2	9.2	9.3	9.0	10.3	11.3
Actinomyces pyogenes	2.6	2.6	2.7	2.6	2.7	2.0	2.4
Yeasts	1.3	1.5	1.6	1.6	1.5	1.7	1.8
Miscellaneous *(Nocardia spp., Pseudomonas spp., Bacillus spp., C. bovis)*	6.2	7.1	6.9	8.5	4.6	6.0	5.8

Samples taken by Udder-Health-Service Extension Workers from Cows with Subclinical Mastitis

Results	1988 N=40 529 %	1989 N=43 628 %	1990 N=38 197 %	1991 N=28 497 %	1992 N=28 116 %	1993 N=29 313 %	1994 N=31 006 %
Bact. negative	**23.7**	**28.8**	**26.7**	**26.4**	**19.1**	**18.0**	**15.4**
Bact. positive (= 100%)	**76.3**	**71.2**	**73.3**	**73.6**	**80.9**	**82.0**	**84.6**
Streptococcus agalactiae	7.2	5.7	5.0	5.6	3.8	3.6	2.8
"Other streptococci" (*S. uberis*, *S. dysgalactiae*, enterococci)	25.1	23.8	24.0	21.9	25.8	27.6	28.4
Staphylococcus aureus "Other staphylococci"	43.3	41.4	42.2	41.4	45.4	45.2	41.5
(*S. epidermidis*,*S. xylosus*, *S. hyicus*, Micrococcus sp.)	16.2	19.0	20.4	16.4	16.5	14.2	18.4
Miscellaneous *(e.g. C. bovis*, coliforms, *A. pyogenes)*	8.2	10.1	8.4	14.7	8.5	9.4	8.9

Prof. Dr M. Schällibaum
Federal Dairy Research Institute, Milk Production Section, CH 3097 Liebefeld-Berne, Switzerland

Events & Meetings

BRITISH MASTITIS CONFERENCE

October 1996

The 9th annual British Mastitis Conference, jointly organized by the Institute for Animal Health, Ciba Animal Health and Genus, will be held on Wednesday 9 October at the usual venue, the National Agricultural Centre, Stoneleigh, near Coventry, UK.

The conference is aimed at everyone with an interest in current developments in mastitis control. It attracts an audience of over 200 veterinary surgeons, advisors, farmers, researchers and milking machine personnel. This year's programme includes papers on antibiotic therapy, both mechanisms and results; practical mastitis control and achievement; research reports; and a session on training and motivation to produce high quality milk.

The conference fee is £42 and tickets are available from J. Reynolds, Ciba Animal Health, Whittlesford, Cambridge CB2 4QT, UK.

1995 Conference

The 8th annual conference again broke records of attendance. The programme was divided into sessions on mastitis problems, the milking machine, mastitis and milk marketing and a research update. In the first session two veterinary surgeons and a farmer compared and contrasted approaches and experience in tackling a high cell count problem and dealing with a farm where coliform infections dominated the clinical mastitis. Next came reports on the commonest milking machine problems found and the effects they could have on milk quality. Two papers considered the demands on milk quality from a purchaser and the legislative intentions of the EU Directive on Milk Hygiene. The final session was the research update and comprised papers on early mastitis detection and early therapy, and an update on progress towards producing a vaccine to control *Streptococcus uberis* mastitis.

Copies of the proceedings, including poster abstracts, are available from J. Reynolds, Ciba Animal Health, Whittlesford, Cambridge, CB2 4QT, UK, price £15 ($25) including UK postage (overseas postage extra). Copies of previous proceedings are also available.

Eric Hillerton

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US NATIONAL MASTITIS COUNCIL ANNUAL MEETING – 1996

The 1996 annual meeting of the National Mastitis Council was held 18–21 February in Nashville, Tennessee. Registration topped 400, with representation from 36 states in the US, Puerto Rico, Belgium, Brazil, Canada, Denmark, France, Germany, Israel, Italy, Japan, Korea, Mexico, Sweden and the United Kingdom. The four-day conference included nine NMC committee meetings, four short courses, a technology transfer session (poster session) and the general session.

General sessions included a session on mastitis control and milk quality in Canada (Dr Ken Leslie), Mexico (Dr Marcelo Perez), United Kingdom (Dr James Booth) and the Nordic countries (Dr Kerstin Plym Forshell). These papers were followed by a presentation, "Is the US milk supply good enough to compete internationally ?", by Mr. Paul Christ from Land O' Lakes, Inc., Arden Hills, Minnesota. A second session dealt with "Milker training and retention on dairy farms". This interactive session was directed by Dr. Robert Milligan, Cornell University and featured the experiences of 3 successful dairy producers from Ohio, Arizona, and Idaho. The third session focused on mastitis pathogens that are not considered to be common but do cause significant problems on some dairies. Papers presented included information on Serratia spp, Mycoplasma spp, Yeast, Prototheca, and a paper on Mammillitis. The final session involved approaches to clinical mastitis management and therapy of clinical mastitis. Proceedings, which include papers presented at the general session and posters presented at the technology transfer session, are available from the NMC office.

In other action at the annual meeting, Dr K. Larry Smith, researcher at the Ohio Agricultural Research and Development Center/The Ohio State University was elected NMC President for 1996–1997. Smith is also currently Chairman of the IDF A2 Group of Mastitis Experts and a member of Permanent Commission A of IDF. The new NMC Vice-President is Dr. Keith Sterner, a veterinarian from Ionia, Michigan. Dr. Sterner will also chair the Program Committee for the 1997 NMC Annual Meeting.

The 1997 NMC Annual Meeting is scheduled for 16–19 February in Albuquerque, New Mexico and will feature a one half day seminar program on Sunday, 16 February, jointly sponsored by NMC and IDF A2. Title for the seminar is "Milk production: hazards and risks from microbial pathogens and chemical residues". NMC will also jointly sponsor a seminar program with IDF A2 in June of 1997. This program will be held in conjunction with the spring meeting of IDF A2 in Guelph, Ontario, Canada and will precede the 1997 meeting of the American Dairy Science Association. The title of the seminar is "Environmental streptococci and mastitis control".

The 1998 NMC Annual Meeting will be held in conjunction with the 4th International Dairy Housing Conference, sponsored by the American Society of Agricultural Engineers (ASAE). The format will be similar to the highly successful 1994 joint NMC/ASAE meeting. The location of this joint meeting which will be held in February, has not been finalized.

Two new publications are available through the NMC. *Procedures for Evaluating Vacuum Levels and Air Flow in Milking Systems* was officially made available at the Milking Systems Short Course held at the NMC Annual Meeting in Nashville. The publication developed by the NMC Machine Milking Committee (Dr Graeme Mein, Chairman) is a step-by-step description of the procedures for complete milking system analysis. Included is a systematic evaluation form for use on the farm, supported by comprehensive explanations and guidelines to ensure the best possible results. The new procedures may be purchased for $5US plus shipping from the NMC office. Forms for on-farm recording of data are available in pads of 50 ($4US each).

Also new from NMC is the 4th edition of *Current Concepts of Bovine Mastitis*. The 4th edition has been completely revised and significantly improved. The cost is $19.95 plus shipping costs.

Plans are underway to develop an NMC home page on the World Wide Web (WWW), providing on-line information dealing with mastitis and milk quality to anyone with access to the Internet. The home page will include basic information about NMC (what NMC is, how to join, etc) along with available publications, meeting announcements, and a list of mastitis experts. The home page will also provide links to other mastitis resources which are already on the WWW such as the IDF Mastitis Research Index (http://www.helsinki.fi/el/tdk/eng/zipengl.html).

In January of 1996 the office of the National Mastitis Council relocated to The New World Dairy Center in Madison, Wisconsin. The new address is: 2820 Walton Commons West Suite 131, Madison, WI 53704 (phone 608/224-0622; fax 608/224-0644; e-mail:nmc@requestltd.com).

All individuals interested in milk quality and mastitis control are encouraged to join the National Mastitis Council. Membership is $50 US for individuals located outside the North American continent and includes the Proceedings of both the Annual Meeting and the summer meeting, Udder Topics (the NMC newsletter published every two months) and a variety of fact sheets on mastitis control. Membership information can be obtained from the NMC office.

Prof. K. Larry Smith

The Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, Ohio 44691, USA

Anne Saeman

Executive Director, National Mastitis Council, 2820 Walton Commons West, Suite 131, Madison, Wisconsin 53704, USA

IDF Publications on Mastitis

All documents listed below can be obtained from IDF Brussels as per address on cover: prices are shown in Belgian Francs.

MASTITIS CONTROL (RESULTS OF QUESTIONNAIRE 1694/A)

by IDF Group of Experts A2 – Bovine mastitis

The replies of 24 member countries to IDF mastitis control questionnaire 1694/A issued in February 1994 are tabulated. The survey shows a high degree of uniformity in recommended mastitis control measures and an increase in their application on-farm since the previous questionnaire 5 years before. There is little evidence of a reduction in infection levels, although cell counts are lower and there has been a big increase in cell count payment schemes in the countries replying to the questionnaire.

Bulletin N°305/1995 – 1400 BEF

TEAT TISSUE REACTIONS TO MACHINE MILKING AND NEW INFECTION RISK

Document prepared by the IDF Machine Milking and Mastitis Subgroup A2D working under the chairmanship of Prof. Dr J. Hamann (Germany)

A description of the physiological status of the teat is used as a reference for the evaluation of the teat tissue reactions induced by machine milking and their impact on the new infection risk.

Bulletin N°297/1994 - 1300 BEF

MASTITIS CONTROL

by a Group of Experts

Results of Questionnaire 1889/A of 16 pages with results from 23 countries: data for cow population, mastitis control schemes, monitoring procedures, antibiotic sensitivity, mastitis control measures, milk payment, progress in mastitis control.

It is part of a three-part Bulletin which also covers payment systems for ex-farm milk and the alkaline phosphatase test as a measure of correct pasteurization.

Bulletin N°262/1991 - 1400 BEF

DESIGN OF CLINICAL TRIALS FOR MASTITIS THERAPY

by Margaret A. Thorburn, Dept. of Population Medicine,Ontario Veterinary College

This 8-page report covers clinical trials of therapeutic treatments; causes of mastitis and its consequences.

It is part of a five-part bulletin which also covers: radionuclides in dairy products; distribution systems for fresh dairy products; enzymes in cheesemaking; and teat & udder cleaning.

Bulletin N°247/1990 - 1500 BEF

MASTITIS RESEARCH INDEX (13TH EDITION, 1996)

MASTITIS RESEARCH INDEX AVAILABLE IN INTERNET

The 13th edition of MRI was published in January 1996. It includes 271 mastitis research projects from 71 laboratories from 28 countries.

The printed version is available from the IDF office (Square Vergote 41, B-1030 Brussels, Belgium, fax +32 2 733 04 13, e-mail: fil-idf@mail.interpac.be).

The index is also available in Internet. If you have access to WWW, use address http://www.helsinki.fi/~hssaloni/. By file transport protocol you can find it at ftp.funet.fi /pub/sci/medical/vetmed/MRI96.zip. The same file is also available as MRI96.txt text file.

The next printed edition of MRI will be published in 1998. Before that the electronic form of the index will be updated, if mastitis laboratories give new research topics. Send the information to the editor by mail, fax or e-mail.

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Mastitis Newsletter N°20

General
- Report of the IDF Group of Experts on Mastitis - J.M. Booth (United Kingdom)
- Hygienic Requirements in International Trade and the Role of Codex Alimentarius and the International Dairy Federation - W.H. Heeschen (Germany)

Research Communications
- Treatment of Mastitis with Homoeopathic Remedies - W.J. Meaney (Ireland)
- Mastitis Cell Count Data - J.M. Booth (United Kingdom)
- Counting Somatic Cells in Milk: International IDF Standard 148:1991 Approved as a Final Standard (1994) - W.H. Heeschen & E.-H. Ubben (Germany)
- Counting Somatic Cells in Milk: Reference Material ("Kiel Standards") - W.H. Heeschen & E.-H. Ubben (Germany)
- The Importance of Coagulase-Negative Staphylococci - K.L. Smith & J.S. Hogan (USA)
- Somatic Cell Counts in Milk of Goats - B. Poutrel (France)

Mastitis notes from member countries
- Finland: The Bovine Udder and Mastitis - M. Sandhol, T. Honkanen-Buzalsik, L. Kaartinen & S. Pyörälä (Editors)
- Germany: New German Guidelines for Mastitis Control - J. Hamann
- Switzerland: Mastitis Pathogens 1988 - 1993 - M. Schällibaum

Events & Meetings
- The 3rd International Mastitis Seminar
- British Mastitis Conference
- US National Mastitis Council Annual Meeting - 1995
- Symposium "Udder Health" in the Netherlands

IDF Publications on Mastitis

Ref. N°142 – September 1995 – 500 BEF

Mastitis Newsletter N°19

Report of the IDF Group of Experts on Mastitis – J.M. Booth (United Kingdom)

Research Communications
- Counting somatic cells in milk: results of IDF intercomparison trials – W.H. Heeschen (Germany)
- Studies on inflammation in the bovine teat with regard to its role in the defence against udder infections – K. Persson (Sweden)
- Cubicle designs for dairy cattle – J. O'Connell & B. Meaney (Ireland)
- Retarded excretion of antibiotics in milk after drying-off therapy – M. Schällibaum (Switzerland)

Mastitis notes from member countries
- Czechoslovakia: Standardization in somatic cell counting – D. Rysánek, V. Babák & L. Slehoferová (Czech Republic)
- Finland: The status of mastitis in the Nordic countries – S. Pyörälä & T. Honkanen-Buzalski (Finland)
- Israel: The national program for the control of mastitis and the improvement in milk quality – A. Saran (Israel)
- Italy: Eradication and control programs – A. Zecconi & G. Vicenzoni (Italy)
- New Zealand: SAMM – A new mastitis control plan – M.W. Woolford (New Zealand)
- Norway: Norwegian cow milk somatic cell count – O. Østerås (Norway)
- Switzerland: Mastitis pathogens 1988-1992 – M. Schällibaum (Switzerland)

Events & meetings

IDF publications on mastitis

Available on request – August 1994

Ref. N°140

Mastitis Newsletter N°18

- Annual report of the IDF Group of Experts on Mastitis (1992) (J.M. Booth, Chairman, UK)
- Mastitis cell count data (J.M. Booth, Chairman, UK)
- Somatic cells in milk - aspects of quality, hygiene & mastitis control (Prof. Dr W.H. Heeschen, Germany)
- Homoeopathic treatment of bovine mastitis (J. Hamann, Germany)
- Cell count interpretation (D.P. Ryan, Australia)

Research communications
- A strategy to increase resistance in dairy cows: expression of human lactoferrin in the milk of transgenic cows (J.H. Nuijens, M. Geerts, R. Strijker, F. Pieper & H.A. de Boer, the Netherlands)
- Systemic dry cow therapy - an update (Dr A. Saran, Dr G. Ziv & Dr S. Soback, Israel)

Mastitis Notes from member countries
- the prevention of mastitis in Italy (G. Ruffo & A. Zecconi, Italy)
- Mastitis pathogens in Switzerland, 1988-1991 (Prof. Dr M. Schällibaum, Switzerland)

Events & meetings

IDF publications on mastitis.

Available on request – April 1993

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MILK - ENUMERATION OF SOMATIC CELLS

Standard/Norme 148A:1995 – 500 BEF