Current Status and Future Challenges in Mastitis Research

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Worldwide, mastitis is one of the most important diseases in dairy cattle. It is important because of the high incidence of the disease and its associated production losses. Moreover, mastitis affects milk quality and has, therefore, consequences beyond the dairy farm. Until recently, mastitis was primarily a concern of dairy farmers and dairy processors. However, because of worries about antimicrobial residues, antimicrobial resistance, milk quality, and animal welfare, mastitis has also become a concern to consumers and society. Udder health has become part of the image of milk: a high quality, healthy and nutritious product produced by healthy animals. Finally, mastitis is the main reason for use of antibiotics in dairy production systems. The importance of mastitis is reflected by the large amount of research on this disease. A search on Web of Science indicated that 242 papers in peer-reviewed scientific journals were published in 2009 using the keyword “mastitis” in combination with “bovine”, “cattle”, or “cow(s)” in the title. Moreover, there is much additional activity in the field of udder health. This year is the 50th anniversary of the annual meeting of the NMC, and therefore we thought it would be appropriate to discuss where we stand in mastitis research. Maybe even more important is to discuss the future direction of mastitis research, given the developments in dairy farming, dairy processing and societal concerns. The paper summarizes the current status of mastitis research, based on topics that were presented at the 5th IDF International Mastitis Conference. Furthermore, some future challenges in mastitis research are defined. Topics evaluated include socio-economics, management, milking equipment, environment, pathogens, diagnostics, therapy, and immunology.

Current Status of Mastitis Research

Socio-economics

Recent calculations for the Netherlands, Sweden and the USA revealed an estimated economic loss from mastitis of $US80 to $125 per average cow on a farm, mostly caused by clinical mastitis. These costs can differ widely between farms as shown for Dutch dairy farms, where the cost estimates for mastitis varied from €17 to €198 per average cow per year. Farmers tend to underestimate these costs.

At the cow-level, a limited number of calculations have been made on cost effectiveness of treatment of clinical or subclinical mastitis, which is very much dependent on transmission of infection between cows. At the herd level, a few studies have been carried out on different types of dry-cow treatment. Recently, studies have been carried out in the Netherlands on cost effectiveness of a large number of preventive measures. Results showed that there is a lack of quantitative data on the effectiveness of management measures, that not all preventive measures are cost effective, and that cost effectiveness depends on the mastitis situation of an individual farm.

For a long time, it was assumed that farmers take a rational approach to the management of mastitis, based on a maximization of the expected benefit. Recently, work in the Netherlands on economics has been set in a broader context. It was found that the economic behavior of farmers is not always rational. Moreover, data showed that farmers are motivated by more than only economics, the most important factor being satisfaction in work. Farmers probably accept that they cannot solve the mastitis problem and will, therefore, remain passive. As a result, it might be that the information on economic consequences will not be considered relevant.

Management

Mastitis is caused by bacteria entering the mammary gland through the teat. Preventing mastitis is based on two approaches: minimizing the presence of bacteria at the teat end, and optimizing resistance of the cow against those bacteria. The first successful attempt to improve mastitis management at the herd level using a planned approach was developed in Reading, UK, in the 1960s. The basic five points in that program were: treatment of all cows at drying off, optimal treatment of cows with clinical mastitis, cull chronically infected cows, optimization of milking and milking machine, post milking teat disinfection of all cows.

This approach mainly focused on contagious udder pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus* which are spread primarily from cow to cow during milking. Even today, in many herds with mastitis caused by contagious pathogens, the five-point plan is the optimal approach to enhance udder health and prevent recurrence. Since the 1960s, the relative distribution of udder pathogens has changed in response to the success of the program. The importance of environmental pathogens such as *Escherichia coli* and *Streptococcus uberis* has increased. Consequently, there is a need for management measures to prevent environmental mastitis problems. At the end of the last century, the NMC extended the five-point plan to a ten-point plan, with 73 sub-points. The ten points are: establishment of goals for udder health; maintenance of a clean, dry, comfortable environment; proper milking procedures; proper maintenance and use of milking equipment; good record keeping; appropriate management of clinical mastitis during lactation; effective dry cow management; maintenance of biosecurity for contagious pathogens and culling of chronically infected cows; regular monitoring of udder health status; and periodic review of the mastitis control program.

In several countries, the ten point plan has been adopted, adapted to the local situation and applied in national programs to improve udder health. Although the basic principles are the same worldwide, differences between areas exist and should be acknowledged. In general, mastitis prevention strategies everywhere comprise more or less the same elements, even though they are not always called as 10-point plan. Local adaptation also has the effect that farmers and advisors feel it is specifically developed for them. This increases their interest in and acceptance of the program.

Milking Equipment

Whatever the system, several basic operating parameters have evolved from research and experimental development. Sufficient vacuum applied to the teat end ranges from 38-46 kPa. A higher milking system vacuum is only warranted when work has to be done on milk, i.e. lifting for transfer. The alternate application of vacuum into the pulsation chamber, to relieve the teat of continuous vacuum and aid circulation has been optimized at approximately one cycle per second with a minimum of 19% of the time within the pulsation chamber at system vacuum. Use of these 'best' parameters minimizes short-term effects on the teat including impaired circulation manifested as cyanosis; medium-term effects such as circulatory impairment resulting in edematous tissue; and long-term effects such as skin erosion or teat canal prolapse. Efficient milking systems aim to limit the duration of any bout of milking whilst ensuring most complete removal of milk possible. The operating conditions thus have to maximize the rate of milk removal, the flow through the teat canal. Flow depends primarily on the diameter of the canal so a higher flow means greater dilation. The greater the dilation the longer it takes for the teat canal to close after milking. As the teat is a visco-elastic structure, the duration of the dilation, the number of cycles (hysteresis effects) also affects the time to 'recovery' i.e. teat canal closure.

Clear evidence exists that machine milking affects the rate of new intramammary infection (IMI). The mechanisms are by aiding contamination of the teat by pathogenic bacteria and facilitating invasion of bacteria into the udder. The former is largely controlled by hygiene and general milking time management. The latter is a product of machine design and the operation of the milking equipment. The modern milking machine is a product of technical advances to create a stable vacuum at the teat end, while minimizing the amplitude of cyclic vacuum fluctuations and avoiding wherever possible irregular vacuum fluctuations. Irregular vacuum fluctuations occur when excessive volumes of air are admitted to the milking system e.g. during cluster change, when clusters fall off and particularly when vacuum reserve is inadequate. Larger volume systems, occasionally with buffer or balance components and greater vacuum supply, have led to a more stable vacuum, and allowed milking to take place at a lower system vacuum. Significantly improved liner designs, better cluster positioning on the cow aided by parlor design and more regular udder conformation have reduced liner slips, thus limiting exposure of teats to high velocity impacts of contaminated milk. This is perhaps one of the most important advances. Cyclic fluctuations in vacuum have been considerably reduced by use of alternate pulsation limiting the amount of air movement when liners open and close. The larger volume liners, wider short milk tubes and higher volume claws mean that air moves more slowly, thus reducing the cyclic amplitude. The modern liner leaves less milk in the udder. Automatic cluster removal (ACR) systems give much gentler treatment of the teat, and lower milking forces reduce rapid air movements, thus impacts. Triggering ACR operation at a higher flow rate without more mastitis supports the hypothesis that a small amount of under-milking is not of major importance, given good hygiene and udder health in general. The adverse effects of over-milking are much clearer, especially on teat trauma. Over-milking can best be prevented by use of ACR. Thus the major benefits for udder health from improved milking machines have come from larger volume clusters with better liner design, alternate pulsation, larger and more stable vacuum systems, wider adoption of ACR and a focus on better teat condition. The latter is significantly aided by understanding that liners deteriorate with age and use. Therefore, liners should be replaced after a fixed number of milkings: 2,500 milkings for a nitrile rubber liner, the most commonly used liner.

In the development of high-tech milking equipment, two directions can be distinguished: 1) high-capacity milking parlors with a high throughput of cows per person per hour, and 2) automatic milking systems in which manual labor is replaced by a milking robot. High-capacity milking parlors are developed in such a way that one operator is able to milk many cows, partly by automation and partly by optimization of available labor. This means that there are only a few seconds available for udder preparation. In an automatic milking system, a robot takes over all manual labor required during milking. Current commercial systems have one robot arm working with one milking stall (one-stall system) or one robot arm working with more milking stalls (multiple-stall systems). With the development of automatic milking systems, the opportunity and need for in-line diagnostics has increased. Diagnosing ill health and abnormal milk will need to be done using technology instead of human endeavor. Combining the information of in-line composite SCC sensing with quarter-based electrical conductivity for the detection of clinical mastitis, produced a success rate of approximately 30% and a false-positive rate of approximately 2%. A number of systems have now been built that utilize this type of information. Automatic milking systems are now available with these types of sensors and also a parlor based in-line laboratory system based on somatic cell count and electrical conductivity (among other parameters). A biosensor has become commercially available for l-Lactate dehydrogenase, an enzyme whose activity is increased because of mastitis.

Along with improved harvesting technology comes the availability of large data sets from individual quarters, cows and herd or parlor performance. Parlor performance data can be used to optimize the efficiency of large milking operations. Large databases are becoming available with udder health data from many cows spanning multiple farms over multiple years. In some situations such databases contain all disease data from all cows in a country. The latter is especially true for the northern European countries. When these large databases become available, analyses of the collected data become important. New statistical software has recently become available to more correctly analyze these large databases.

Environment

The environment affects all aspects of dairy husbandry required to produce quality milk in a profitable management system. Basic among environmental factors dictating the prosperity of any biological species is the control of population density within the context of food availability and waste disposal. Overstocking of cattle and lax manure management will exacerbate the detrimental effects of the environment on mastitis control. Most dairy systems have a reasonable control of ensuring adequate nutrition to cows in either grazing or stored forage-based feeding systems, but the influence of manure management on health of cows has been poorly defined. The common themes for reducing udder pathogens in the cows' environment are controlling fecal accumulation, reducing moisture, and minimizing organic contamination. These factors are consistent across total confinement free-stalls, grazing, dry lots, tie-stalls and any combination of systems a producer may use on their farm.

Environmental udder pathogens are often of fecal origin and cannot live on teat skin for long periods of time. If these bacteria are present in large numbers on teat skin, it is the result of recent contamination. Therefore, the number of these bacteria on teat skin is a reflection of the cow's exposure to the contaminating environment. Total confinement housing systems often increase pathogen load in the cows' environment compared with grazing management systems. A significant source of udder pathogens in total confinement systems is the material used for bedding cows either in stalls or loose housing. Organic bedding materials such as straw, wood products and recycled manure commonly contain few udder pathogens prior to use as bedding. However, these organic products rapidly become contaminated, with the mastitis pathogen populations increasing 10,000-fold within 24 hours. Efforts to control pathogen populations in organic beddings by composting or the use of sanitizing and disinfecting agents have been unsuccessful. Inorganic bedding, such as sand, supports lower bacterial populations compared with organic bedding. The bacterial contamination of sand bedding is directly related to the moisture and organic contamination. Reclaiming and recycling of sand often leads to higher organic content of bedding and greater exposure to udder pathogens compared with fresh sand. The exposure of cows to udder pathogens in pasture-based systems is largely dependent upon stocking rate and plant coverage of the soil. Management practices resulting in barren soils - in maternity pens, loafing areas, paddocks and cow races - can expose cows to greater populations of udder pathogens than those in organic bedding materials. Bacterial populations of environmental udder pathogens can persist for up to two weeks after intensive grazing is discontinued on pastures. Research has indicated that a quiescent period between rotation of cows on paddocks is needed to allow forage regrowth and dissipation of the manure load.

The environment and physiology of dairy cows interact to affect mastitis and milk quality. Rates of new IMI caused by environmental pathogens are greater during involution and lactogenesis than during lactation. Pathogen exposure and subsequent rates of mastitis during the dry period are increased by use of stalls and maternity housing areas bedded with moist organic bedding. Manure packs generally contain extremely high counts of pathogens dangerous to both dam and calf. Maternity paddocks in seasonal and grazing-based systems are often mud and manure laden soil exacerbated by calving season and rains. Decreasing cow density, using inorganic bedding, and frequently cleaning maternity areas reduces pathogen exposure compared with crowded organic bedding packs and soil-covered paddocks.

Pathogens

A large number of microorganisms have been reported to cause bovine mastitis. Most of those are bacteria, but fungi and algae may also cause mastitis problems in some herds or regions. Worldwide, however, the most common udder pathogens are staphylococci (*S. aureus* and several coagulase-negative staphylococcal species (CNS), streptococci (*S. agalactiae, Streptococcus dysgalactiae, S. uberis*) and coliforms (*E. coli*, *Klebsiella* spp), even though other pathogens, e.g. *Mycobacterium bovis*, may cause problems in some regions. The relative importance of the pathogens has varied over the years, and does still vary between countries, regions and farms, mainly due to differences in management and housing systems.

S. aureus causing contagious mastitis remains an important mastitis pathogen in most countries. Antibiotic treatment plays a limited role in the elimination of *S. aureus* mastitis, as many infections are hard to cure. *S. aureus* is generally a clonal organism as the populations in dairy herds often consist of groups of genetically related strains. Strains originating from bovine mastitis mostly represent a genetically different cluster than e.g. human strains, suggesting host-specificity. *S. aureus* can produce numerous putative virulence factors that allow adhesion to membranes and resistance to phagocytosis. Studies on bovine mastitis strains suggest that virulence of *S. aureus* can vary among strains.

Mastitis due to coliform bacteria, mainly *E. coli*, is an increasing problem in many countries. *E. coli* mastitis is common in high-producing cows with a low milk somatic cell count. In *E. coli* mastitis, severity of clinical signs is considered to depend mainly on the host response. The effective elimination of the bacteria by neutrophils is important for the resolution of infection. If delayed, the disease can lead to development of toxemia and septic shock. *E. coli* isolated in mastitis are generally a very heterogeneous group. The bacterial strain, however, may also have a role in the pathogenesis. Horizontal transfer of genes is typical for *E. coli* which may result in evolution of new, pathogenic clones. Evolution of mammary-pathogenic *E. coli* strains has been proposed and is supported by the presence of persistent *E. coli* IMI.

Mastitis caused by *S. uberis* seems also to be increasing, and prevention strategies against mastitis caused by *S. uberis* are often unsuccessful. *S. uberis* can produce a persistent infection that often is unresponsive to treatment. It is known that *S. uberis* exploits host proteins secreted into milk to establish a successful IMI. *S. uberis* is mainly an environmental mastitis bacterium, but recent findings suggest that it may also behave as a contagious pathogen.

S. agalactiae has been considered more or less non-existent in many areas, but recent findings indicate that this pathogen is re-emerging, at least in some countries. The importance of this pathogen is emphasized by the fact that the same genotypes have been identified in human and bovine samples.

CNS have gained increasing interest, and are among the most prevalent udder pathogens in some countries. Knowledge on the relative importance of different CNS species is also gathering, which may result in the need for differential diagnoses and different control methods depending on species. Following the increasing importance of CNS and greater knowledge of the importance of different CNS species, the need for accurate species differentiation of CNS may grow. Biochemical species differentiation is very time-consuming and sometimes difficult to interpret. Therefore, several genotyping methods for differentiation have been introduced but may still be too costly for routine diagnosis. Common guidelines/recommendations on which genotyping method/s to use would make comparisons between studies easier.

To understand why some species/strains are more infectious, contagious and/or more resistant to treatment than other species/strains, knowledge on specific virulence factors is needed. Also in this area genotyping is an important tool. Some progress has been achieved in the understanding of important virulence factors for some pathogens.

Diagnostics

As treatment and control programs vary depending on the characteristics of the udder pathogens, correct microbiological diagnosis is essential. To achieve this, the sampling procedures and sample handling is important. Even though some basic methods, such as the NMC Handbook and IDF Bulletins, are widely accepted, there are still uncertainties about the best routine to use in specific situations, for example number of colony-forming units to be defined as positive, quarter milk or composite milk sampling, and the number of samples needed to determine if a quarter/cow/bulk tank is positive or not. Another important factor is the transportation of milk samples to the laboratory. Chilling of the samples is an important recommendation, but practical and economic solutions on how to achieve this in different situations are needed. Studies on the effect of freezing milk samples on bacteriological results have also given varying results.

The next essential step towards correct microbiological diagnosis is good laboratory technique. Today bacteriological culturing, in combination with confirming biochemical methods, is routinely used. Such methods may be time consuming, difficult to interpret, and rely heavily on skilled and experienced personnel. Detailed guidelines on inoculation volume and biochemical methods are available, but not on interpretation of growth which may vary between laboratories. In certain situations, for example, decisions on treatment of acute clinical mastitis, rapid field and/or cow-side tools are warranted.

Recent studies on the diagnostic validity of on-farm culture systems (selective agars, petri-film and tri-plates) show an acceptable performance, although specificity is relatively low, indicating many false-positive diagnoses. It can be concluded that such tests are able to appropriately categorize cases of mastitis under farm conditions. This would potentially result in a more precise use of antibiotics to treat clinical cases of mastitis.

The genomic sequences of many of the major mastitis-causing pathogens are now available and have been utilized to develop nucleic acid-based testing methods, such as PCR technology. Currently, PCR-based tests are generally more expensive than classical bacteriology. However, when they prove to be sufficiently sensitive and specific and can be performed rapidly, these techniques may complement or eventually replace culture-based diagnostic tests. PCR tests allow the identification of closely related organisms within a few hours. A multiplex PCR, 'real-time' PCR assay that can simultaneously detect different mastitis-causing organisms in milk samples is already commercially available in a number of countries. Molecular diagnostic methods are not only useful for the diagnosis of mastitis organisms, they may also help to identify particularly virulent strains of an organism or distinguish between clonal and non-clonal infection outbreaks. In a clonal outbreak, the observed predominance of a single strain could indicate contagious transmission of the organism or exposure of multiple cows to an environmental point source. Putative virulence genes in *S. aureus* that are identified e.g. in persistent and non-persistent bovine IMI, could also be screened. Strains possessing *sed*, *sej*, and *bla*Z genes, often in combination with penicillin resistance, have been typically found in connection with persistent IMI. This finding, that *S. aureus* strains causing persistent IMI can be identified based on their genetic makeup, may be utilized for strain-specific diagnosis.

Therapy

Targeting treatment towards specific pathogens when possible is considered necessary for rational antimicrobial treatment of mastitis, as should be the case in all treatment of bacterial infections. It is clear that mastitis caused by Gram-positive agents needs different approaches than mastitis caused by Gram-negative bacteria. Because new diagnostic tools such as PCR-based tests and selective agars have made rapid identification of mastitis-causing bacteria feasible, routine use of broad-spectrum antimicrobials without diagnosis could be considered as an outdated practice. There is evidence that the duration of treatment should be adjusted by pathogen, as infections caused by certain bacteria need longer treatment. It is clear that the current 1-2 day routine treatments are generally not efficacious. Efficacy of supportive treatment such as non-steroidal anti-inflammatory agents, frequent milking and fluid therapy in mastitis has been studied, in combination with antibiotic treatment. Use of non-steroidal anti-inflammatory drugs has been shown to be beneficial at least in clinical mastitis. Herbal or homeopathic remedies have not been shown to be effective for mastitis treatment in several independent, scientific studies.

Development of resistance after antimicrobial treatment of mastitis is an important consideration, even though the proportions of resistant isolates have generally not yet been at an alarming level. Full consensus about the value of in vitro susceptibility testing in mastitis treatment is still lacking. Some studies have shown no correlation between in vitro susceptibility of the causative pathogen and cure rates. To reveal the predictive value of in vitro susceptibility testing, studies should meet certain requirements including that breakpoints used should reflect the pharmacokinetics and pharmacodynamics of the drug i.e., concentrations maintained in the udder in relation to the MICs of the bacteria. Treatment should be long enough for the elimination of the infection to be possible, and a control group to show spontaneous recovery rates should be included. In mastitis caused by *S. aureus*, penicillin resistance has resulted in significantly lower cure rates compared with infections caused by susceptible isolates.

Blanket dry cow therapy for healthy cows is not practiced in all countries. It may have its disadvantages and certainly adds to antibiotic consumption. The non-antibiotic approach for dry cow therapy, an internal teat seal, is a good alternative to antibiotics in herds with a high risk for environmental mastitis during the dry period and at calving. The use of non-antibiotic teat seals for cows at risk at drying-off is now a well-established practice in many herds world-wide. Pre-partum treatment of heifers with antibiotics has been reported with some positive results. It is unfortunate that in the studies on pre-partum antibiotic use in heifers, the justification of routine use of antimicrobials is generally not discussed, nor the possible impact of this practice on the development of antibiotic resistance.

Immunology

Two critical factors related to the risk of mastitis are the immune competence of the animal and exposure to pathogens. Immune competence of dairy cows is influenced by several factors including stage of lactation, breed, genotype, age, milk yield, energy balance, plane of nutrition, and pre-existing infections. Exposure of the udder to pathogens can occur from pathogens in the environment of the dairy cow and from infected animals.

Immunity of the bovine mammary gland is mediated by non-specific defenses often referred to as innate immunity and by specific defenses referred to as acquired immunity. Innate immunity predominates during early infection, is activated quickly by numerous stimuli, is not augmented by repeated exposure, and can eliminate bacteria without altering milk quality. Innate immunity involves nonspecific resistance factors such as anatomy (e.g. teat canal), phagocytic cells (e.g. polymorphonuclear neutrophils and macrophages), and soluble factors (e.g. lactoferrin, cytokines, and complement). Acquired immunity is important when innate defenses fail. During acquired immunity, specific antigens are recognized and the immune response is heightened upon repeated exposure. Acquired immunity is the basis behind vaccination strategies which attempt to stimulate T- and B-lymphocytes and macrophages, and the production of specific antibodies.

The developing adaptive immune response is a complex interaction between the host and pathogen where dose, strength, and duration of exposure all combine to direct the immune response. The adaptive immune response centers on activation of CD4+ T-lymphocytes which help orchestrate subsequent immune responses through release of cytokines. There are several CD4+ subfamilies currently recognized. Two dominant CD4+ T helper lymphocyte families, Th1 and Th2, affect immunity to both intracellular (primarily Th1) and extracellular (primarily Th2) pathogens. The Th1 response drives cell-mediated immunity to kill infected cells while the Th2 response drives humoral or antibody-mediated immunity to limit infection. Two important questions exist: 1) which arm of the immune response is more protective against subsequent infection, and 2) which adjuvant is more effective in inducing a Th1 compared with a Th2 response. Interferon-γ and interleukin-4 are generally limited to either CD4+ Th1 or Th2 lymphocyte subsets, respectively. Interferon-γ also leads to class switching to the IgG2 isotype which is more efficient at opsonizing antigen and increasing phagocytosis by neutrophils which have surface receptors for IgG2. In contrast, interleukin-4 promotes class switching to IgG1 which is less effective at opsonization.

Cell-mediated immunity promotes monocyte/ macrophage killing via release of IFN-γ and other cytokines. The timing and balance of cytokines produced by cells in the local environment help provide this direction. Evaluation of targeted cytokine profiles including interleukin 1β (IL1β), IL4, IL6, IL10, IL17A, and interferon γ has provided insight into the impact of vaccination on the adaptive response, subsequent resistance to infection, and guidance for future vaccine formulations against some important udder pathogens. In addition to targeted cytokine profiling, isotype-specific antibody responses are involved in a variety of functions including inhibition of mastitis pathogen adherence to and internalization into mammary epithelial cells.

Different cells in the mammary gland such as dendritic, natural killer, γδT, endothelial, mammary epithelial, neutrophils, macrophages, CD4+ and CD8+ T cells and IgA-producing plasma cells play an important role in local inflammatory responses. Macrophages are the dominant cells in milk, dry secretions and tissues of healthy udders. They also phagocytose and kill bacteria using proteases and reactive oxygen radicals. Macrophages secrete IL-12, which potentiates development of IFN-γ secreting cytotoxic CD8+ cells. However, they are fewer in number during mastitis. The main effectors of mammary gland immune defenses against major mastitis-causing bacteria are blood derived neutrophils, opsonizing antibodies and effector-T cells in cooperation with cytokines. Neutrophils are actively recruited to the site of infection and are the major cell type present in the mammary gland during the early phases of mastitis. After access to the mammary gland, most udder pathogens grow very rapidly and quick recruitment of fresh blood neutrophils and opsonizing antibody of IgG2 isotype is required. These recruited neutrophils require bacteria opsonized by IgG2, IgM, or C3bi/C3b for efficient clearance of infections. In contrast, CD4+ Th2 lymphocytes lead to release of interleukin-4 and -5 which promotes an IgG1 class switch.

Alterations in the Th1 compared with the Th2 response can have a direct effect on the functional ability of macrophages and neutrophils, the dominant cell type in most infected mammary glands. Macrophages can bind both IgG1 and IgG2 to promote opsonization, but one response may be more effective than the other. In contrast, neutrophils which arrive in large numbers early in infection, primarily express IgG2 Fc receptors. Thus, neutrophil opsonization is improved when IgG2 concentrations are greater. Cell-mediated immunity (a Th1 dominant response) can also enhance the killing ability of macrophages and other cells such as natural killer cells through release of IFN-γ and other cytokines.

Future Challenges

Socio-economics

Although farmers perceive mastitis as an annoying problem and intend to have a mastitis incidence as low as possible, usually they are not motivated to change their farm management. Such intention-behavior discrepancies have rarely been studied in veterinary medicine. More insight in this field is necessary to know how to motivate farmers to change animal health and welfare management in a way that is advantageous for the sector as a whole.

Economic calculations have been made at the cow and herd level. All of these calculations are model-based and not data-based. At the cow level, calculations are done on treatment and culling decisions. These calculations are dependent on results of clinical trials. Good quality clinical trial data are not available from enough studies e.g. on longer treatments, which means that cure rates may be based on rough estimates instead of good data. One positive and very important effect of improved cure and prevention is the reduction in incidence of new IMI. The benefits have only been studied in simulations and the proof should come from a clinical trial at the herd level. Although there is much expert knowledge on the effectiveness of management measures, there is not much quantifiable knowledge. This gives a high level of uncertainty to cost-effectiveness calculations. Finally, there are no known calculations of the effect of mastitis on sector level. Whole sector economic calculations, including modeling of the effect of image of dairy farming on demand for milk and milk products, should be carried out to be able to evaluate the cost-effectiveness of mastitis prevention programs at the sector level.

Management

Worldwide, there are a number of mastitis control programs in place. These programs all have a somewhat different approach and some require a very high level of management and involvement by dairy farmers. The effectiveness of these programs in terms of changed intentions, changed behavior, change in mastitis situation and cost-benefit has not been studied extensively. Another missing factor is knowledge on how farmers can be motivated to apply mastitis control programs. This requires knowledge from the field of communication. Many advisers, veterinary practitioners and others, do have the technical knowledge available, but are unable to motivate their clients to use that knowledge to prevent mastitis, rather than acting merely reactive when problems do occur. Local situations on personnel management are very different, ranging from a 25 cow farmer in Norway to a 2000 cow herd manager with Spanish speaking milkers in North America. More insight on cultural differences between various countries in relation to the motivation of farmers to implement mastitis prevention programs should be studied.

More emphasis is necessary on the role of the second line of defense. Although there is much to be learned in the fields of natural and acquired host resistance, much knowledge is already available, which should be implemented in mastitis prevention programs. With an increasing number of automatic milking systems in some parts of the world, the need for mastitis prevention programs for herds using these systems is growing. Although many factors do not differ from regular milking herds, some certainly do, causing a need for specific prevention programs.

In future programs, there is a need to incorporate modern communication technologies. More work in that field is needed and, although some general principles can be applied, these programs need to be executed locally for full benefit to be achieved.

Milking Equipment
Future developments should focus on new technologies of automatic milking systems, and other ways of milking teats individually. Introduction of sensors into the milking cluster must not disrupt the best milking operation, swift and complete removal of milk whilst also maintaining the integrity of milk and the health of the teat. An open teat canal is the primary risk factor for environmental mastitis. Thus far, very little direct attention has been paid to managing the teat canal other than post milking teat disinfectants and keeping cows standing after milking. Future evaluations of performance of the machine milking should include teat closure.

New sensing technologies are available to identify udder health problems in cows or in herds earlier and more accurately. There is an important need to evaluate the sensors under field conditions and to implement algorithms to improve the performance of these technologies to fulfill the needs of dairy farmers. To date, most studies on new sensing technologies have focused on technical characteristics and capabilities of the systems. However, the value of these technologies lies in improved information that may result in better and faster interventions by the dairy producer. Also, many of the proposed technologies are necessarily associated with increased operation costs and a technologically advanced infrastructure. Further studies on the value of detection equipment in precision agriculture need to be conducted.

Environment
A basic understanding of many environmental factors affecting bacterial exposure and host resistance is still lacking. Based upon susceptibility to new IMI by mastitis pathogens, the ranked priority areas to concentrate research for reducing exposure to environmental pathogens are the periparturient, recently dried-off, pregnant heifer, lactating, and dry cow environments. Empirical trials are needed to determine appropriate cow density for various management systems. The effects of frequency of manure removal on pathogen exposure have not been defined. Alternative bedding sources that reduce pathogen exposure, but are ecologically suitable, need to be sought and researched. Future environmental mastitis management plans must be coordinated with meeting cows' nutritional needs, ensure positive behavioral and social interactions among animals, and stabilize the ecology on the farm and surrounding communities.

Pathogens
To further understand the pathogenesis of infections, the interactions between bacteria and host, including the interaction between genotype and environment, requires further study. Understanding of the epidemiology and pathogenesis of several common udder pathogens has

increased markedly. However, additional knowledge is still needed (e.g., on factors important for infection and spread). Moreover, relatively little is known about THE epidemiology, ecology, pathogenesis and strain variation of the important staphylococcal species and environmental streptococci (*S. uberis* in particular but also *S. dysgalactiae*) important udder pathogens in many countries and herds. The re-emergence of *S. agalactiae* also needs further investigation.

Diagnostics

Correct identification of infected cows is the basis for progress in mastitis control and treatment. This is especially true for contagious udder infections. Thus, simple, low-cost and accurate sampling and diagnostic procedures designed for specific scenarios are needed. Depending on the prevalence of different species and/or strains, such scenarios may differ between countries, regions or farms. Possible differences between strains within species in optimal sampling and diagnostic procedures should also be taken into consideration.

With the availability of new and faster diagnostic methods, reporting of results to the dairy producer is becoming more important. To allow the producer to use rapid bacteria-specific diagnosis for treatment or other interventions, results should be made available instantaneously. Reporting in many diagnostic laboratories is now via email and through directly accessible web-based systems. However, these methods still require transcribing of diagnostic results into on-farm management systems. In some situations, diagnostic test results are now made available real time and directly into the on-farm software.

Therapy

Research into treatment of bovine mastitis is quite traditional and seldom contains new approaches. Pharmacological and pharmacodynamic aspects should be considered more, as well as prudent use guidelines and risk of development of antimicrobial resistance. A weakness in many field studies on mastitis treatment is that results are not presented per bacterial groups, nor is in vitro susceptibility of the causative agents to the antibiotic reported. Unfortunately, many of the published mastitis trials do not meet standards for good quality clinical trials. To produce conclusive results, treatment groups in mastitis trials should be randomized and balanced, and confounding factors taken into account in statistical models. As milk is used for human consumption, antibiotic residue aspects should always be taken into consideration and discussed. Also, when cost-benefit studies are carried out, selection pressure towards antimicrobial resistance should be taken into account as a cost factor. Because of increasing societal pressure on use of antibiotics for disease prevention, the concept of selective dry cow therapy and use of non-antibiotic strategies deserves more attention.

Other points to consider include bacterium-related factors affecting treatment response, targeting treatment according to the causative agent, comparison of systemic versus intramammary therapy, application of supportive treatment and an optimal duration of treatment for different IMI. Farm level trials in different conditions are necessary to explore economic benefits of treating different types of mastitis. New treatments, possibly non-antimicrobial, will be welcome. Evaluation and elimination of pain and discomfort in mastitis and the efficacy of pain-relieving treatments should be studied also under field conditions.

Immunology
Approaches to enhance the cow's immunity to prevent disease and thus minimize use of antibiotics have gained considerable attention. Yet, for a variety of reasons, vaccines developed for the prevention and control of mastitis have achieved only limited success. The multiplicity of pathogens capable of causing mastitis, and insufficient knowledge of mammary gland immunology, bacterial virulence factors, and mechanisms of pathogenesis are factors that have hindered development of effective mastitis vaccines.

Control of pathogens using immunological approaches requires identification of virulence factors and elucidation of their role in the pathogenesis of the infection that they cause. Equally important steps are validation of antigenicity in vivo and ubiquity of the virulence factor among strains of the pathogen. The availability of completely sequenced genomes of several pathogens allows elucidation of amino acid sequences and identification of dominant epitopes of newly discovered virulence factors. Use of this information, coupled with the availability of in vivo experimental approaches to test immune responses, vaccination timing, and protection against experimental infections will undoubtedly result in identification of virulence factors to be used in the design of vaccines for better control of mastitis and other diseases of food producing animals. Furthermore, identification and characterization of specific genes involved in mastitis resistance/susceptibility could result in new approaches of mastitis control through genetic selection. Additional research is needed to identify cow T- and B-lymphocyte response factors in relation to vaccination and experimental challenge that will provide direction for designing future vaccine formulations and therapeutic strategies.

Concluding Remarks

Advances in mastitis research in the last decade or so have brought exciting new knowledge and technologies that can/will be used to solve complex problems confronting dairy production. These advances could have a fundamental impact on dairy production as we now know it. For example, improved disease surveillance and enhanced disease resistance are just a few of the possibilities that could have an impact in the not-too-distant future. A better understanding of the multiplicity of pathogens capable of causing mastitis, and knowledge of mammary gland immunology, bacterial virulence factors, and mechanisms of pathogenesis will facilitate development of effective mastitis vaccines. New developments, approaches, strategies, and advances in mastitis diagnosis, treatment, and prevention can dramatically improve dairy herd health programs and result in reduced severity of mastitis, increased production and profitability of dairy farms, and ensure a supply of safe and nutritious dairy products for consumers throughout the world. It is for today's researchers to take up that challenge.

References

An extensive list of references can be obtained from the authors.