Mastitis is the most prevalent pathological condition seen in dairy cows. It affects, with varying degree, the world dairy herds and accounts for the majority of antibiotic use in the dairy industry. This disease leads to important economic losses in dairy herds due to decrease in production, discharge of milk unfit for human consumption, treatment costs (drugs, veterinarian fees, labour), early culling, and mortality of affected individuals. In Canada, we estimate total losses at more than 400 million dollars per year, representing approximately 15% of the industry’s net income.

The Canadian Bovine Mastitis Research Network (CBMRN) was conceived in 2001 to directly address this economically important disease of the dairy industry. The CBMRN is primarily a research network and its membership consists of over 40 researchers and close collaborators from universities and research centers in Canada, the United States, and in other countries. The CBMRN research program objectives focus on the priorities and needs of the Canadian dairy industry:

- Define the mastitis problem in Canada;
- Conduct research to elucidate new mastitis solutions;
- Transfer new mastitis knowledge to dairy producers and veterinarians;
- Establish a foundation for networking to perpetuate research and transfer activities in the future.

Financially supported by the Canadian dairy industry, Natural Sciences and Engineering Research Council of Canada (NSERC), and other valuable partners, the CBMRN has undertaken in 2006 an ambitious research program to respond to those priorities. After two years of active investigations and collaboration, we are proud to present this first reference booklet describing the Network research results. This document presents the “Who, What, Where, and How” of research projects and their “artisan”.

The Canadian dairy industry and the CBMRN are striving to create value for the industry and for society by reducing mastitis and maintaining milk quality. The CBMRN’s role is to supply knowledge and technology toward these objectives. The industry’s role is to implement the knowledge and technology on-farm. This is now the time, for you, to take part in this role…

www.mastitisnetwork.org
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Who, What, Where, How...

The research program of the CBMRN is centred on the Core Research Platform, a hub of knowledge and data that links our two research themes: Mastitis Monitoring and Mastitis Control. The Mastitis Monitoring Theme’s objectives are to develop knowledge and technologies for detecting and tracking the occurrence of mastitis and intramammary infection. This is accomplished by optimizing the interpretation and use of existing monitoring information, and by integrating new ideas into established mastitis management practices. Objectives of the Mastitis Control Theme are to enhance the cow’s resistance to mastitis pathogens and to develop therapeutic strategies to control this disease. Evaluation of the association between mastitis treatment and bacterial resistance to therapy is also part of this theme.

1 - National Cohort of Dairy Farms

The NCDF is composed of 91 dairy farms spread across six provinces. This cohort enables and harmonizes mastitis research by providing to a diverse group of scientists uniform data that originates from a single, nationally representative population of dairy farms. Uniform protocols are implemented for repeated quarter milk sampling of clinical mastitis cases, randomly selected non-clinical lactating cows, and on a selection of cows at dry-off and after calving.

CBMRN students and technicians sample milk, blood, and other biological material in their respective province for the Network’s research.
program. It links mastitis researchers with dairy farmers. The Core research platform consists of the National Cohort of Dairy Farms (NCDF), the Mastitis Pathogen Culture Collection (MPCC) and the Mastitis Laboratory Network (MLN).

2 - The Mastitis Pathogen Culture Collection

The MPCC is the CRP vehicle that provides access to bacterial pathogens isolated from udder and bulk tank milk samples. Milk bacteriology results are recorded in a central database and the bacterial isolates are archived in a centralized culture collection. Isolates are readily available to CBMRN researchers and play a critical role in Network projects which investigate pathogen virulence factors and antibiotic resistance. A particular strength of the MPCC is that isolates are fully cross-referenced with all NCDF data: epidemiologic and demographic data associated with cows and farms, physiology data, and archived DNA samples of cows. Over eighteen thousand bacterial isolates have been conserved in the MPCC to date.

3 - The Mastitis Laboratory Network

The MLN is an assembly of diagnostic laboratories across Canada that utilize standard operating procedures for the identification of bacterial pathogens in milk samples. The MLN receive and culture all milk samples from the NCDF, identifies and records bacterial pathogens in those samples, and sends isolated bacteria to the MPCC. The milk sample records from the MLN are submitted to regional coordinating centres for the reporting of culture results to participating farmers and their veterinarians, and for entry into the CRP database. Over ninety five thousand milk samples have been processed and recorded thus far.

In addition to the MLN laboratories at the University of Prince Edward Island, University of Saskatchewan, and the University of Montreal, work is progressing to establish a milk quality diagnostic laboratory at the University of Calgary during summer 2008.
Overview of Methods

- 106 dairy herds in all 10 provinces of Canada were purposely selected by local veterinary practitioners or provincial Canadian Quality Milk Program coordinators;
- Production and Somatic Cell Count (SCC) data were provided for all herds. Specific cow and lactation data (calving dates, parity, and culling dates) and specific herd data (Bulk Milk SCC (BMSCC) and herd size) were obtained from DHI;
- A questionnaire was administrated on every farm;
- Producers were asked to aseptically collect a milk sample from every quarter that had visible signs of clinical mastitis and to record cow identification, quarter, date, and clinical signs;
- Bacteriological culture of milk samples was performed according to NMC standards.

Summary of Findings

- Overall mean IRCM was 23 cases per 100 cows per year ranging widely among selected herds - the most frequently isolated pathogens from clinical mastitis were Staph. aureus, E. coli, Strep. uberis, and coagulase-negative staphylococci;
- Tie-stall barns had the highest Staph. aureus, Strep. uberis, and coagulase-negative staphylococci;
- Compared with Western provinces, participating herds in Ontario and Quebec had the highest mean herd IRCM, possibly associated with the predominant barn type in those regions being tie-stalls. E. coli IRCM was relatively higher in Ontario than in other regions, but Strep. dysgalactiae IRCM was highest in Québec;
- Herds in free-stalls had lower Strep. uberis IRCM;
- During lactation, IRCM was highest in the first week after calving;
• There was no association between BMSCC and overall IRCM, but \textit{E. coli} and culture-negative IRCM was highest in low and medium BMSCC herds;

• Several risk factors were associated with overall and pathogen-specific IRCM. For example, blanket dry cow treatment was associated with decreased overall IRCM. Attitude risk factors, such as writing down milking procedures, were also associated with lower IRCM;

• Checking first streams of milk were associated with higher overall IRCM and more specifically with \textit{Staph. aureus} and \textit{Strep. dysgalactiae} IRCM, because more clinical mastitis will be discovered.

\textbf{Take Home Message}

1 - Mastitis prevention and control programs should differ among regions and be tailored towards housing type and BMSCC. In this study, especially in tie-stall herds, only 50\% of milkers wear gloves during milking.

2 - Mastitis control programs in Canada should take into account the producers’ attitude towards mastitis management practices. Checking first streams of milk is a good practice for early detection of clinical mastitis.

3 - Pathogen-specific risk factors can be quite different, and it is therefore important in mastitis control programs to identify the pathogen that causes problems in a herd to better target therapy. In this study, only 15\% of the dairy herds cultured milk samples.

\textbf{Preventing Transfer of Pathogens Among Cows During Milking}

Many different techniques can be used to control pathogen transfer. The main idea is to do nothing that will pick up bacteria from one cow and move it to another cow. Single use towels (paper, cloth, wipes) should be used in preparation of the udder and teats. Sponges and common rags are prohibited. Gloves for milkers are helpful and should permit constant washing without irritating the hands. Separate milking units for infected cows, established milking orders to protect the non-infected cows, and hospital pens with separate milking equipment are all appropriate methods. Post-milking teat dipping is very important. The more the teat is covered, the more effective the teat dip will be. Dipping with a cup is usually more effective than spraying.

Assessment of the Mastitis Situation in Canada
Part 2 - Management Practices Associated with the Bulk Milk Prevalence of Contagious Mastitis Pathogens in

Overview of Methods

- 289 herds were randomly selected from herds that participated in DHI recording in 2003 - more herds were recruited in Ontario and Quebec because these provinces have a larger population of dairy herds than other provinces;
- For each herd, production and SCC data were collected from DHI organizations;
- 4 bulk milk samples per herd over a period of 1 year were collected by milk haulers and sent frozen to the Atlantic Veterinary College, PEI, for bacteriological culture;
- All participating producers were asked to fill out a questionnaire focusing on mastitis prevention.

Summary of Findings

- *Staph. aureus* is present in bulk tank milk of nearly all Canadian dairy farms (Figure 1) - the highest prevalence was found in Saskatchewan (90%), followed by the Atlantic provinces (86-88%);
- No *Mycoplasma* spp. were isolated (probably due to freezing sample - found 2% on fresh samples from PEI);
- *Strep. agalactiae* may be, except in Quebec, at the brink of extinction in Canada (less than 1% of the bulk milk samples);
- The province with the lowest BMSCC, British Columbia, also had the lowest prevalence of *Staph. aureus* in bulk milk;
- Adoption of most of the recommended mastitis management practices is high in Canadian dairy herds;
- Certain management practices were practised more often in free-stalls than in tie-stalls: pre-milking teat disinfection, wearing latex gloves during milking, vaccinating cows for mastitis, using a computer for herd management;
- In tie-stalls, mastitis cows were more often milked last or with a separate cluster and bedding was more frequently changed (90% of tie-stalls use straw as primary bedding material);
- Blanket dry cow treatment, believing that a nutritionist is important in mastitis data review, feed company nutritionist balances the ration, and the ration is balanced at least twice a year were associated with a lower probability of isolating *Staph. aureus* from the bulk milk.

Figure 1. Prevalence of *Staphylococcus aureus* in Canadian dairy herds, by province.
Take Home Message

1 - Significant improvement of the mastitis prevalence and incidence can only be achieved if herds monitor the mastitis situation within the herd. Permanent record keeping and review of the data together with a specialist are essential in this respect. Use of readily available data such as BMCSS and DHI SCC is not sufficient for this purpose.

2 - Because the effect of prevention and control measures is different for the pathogens involved, determination of the distribution of pathogens involved in subclinical mastitis cases on a regular basis is also necessary. Let’s culture milk!

3 - Only a small proportion of the Canadian dairy farms samples clinical mastitis cases. Farms that don’t do it essentially implement mastitis prevention practices without knowing what the target pathogens are. The general assumption is that the rate of antimicrobial resistance is closely associated with the rate of antimicrobial use.

4 - Improvement could be achieved in Canadian dairy farms by:
   - Practicing blanket dry cow treatment (practiced in only 72% of dairy herds);
   - Wearing gloves during milking (especially in tie-stall herds where only 50% wear gloves);
   - Increasing implementation of the NMC plan for the control of mastitis (only 43% of farms implement all points).

5 - The Canadian mastitis control program should:
   - Focus on reducing *Staph. aureus*;
   - Focus on information transfer about recommended management practices;
   - Find ways to motivate producers to implement these practices – establish annual goals.

10 Points NMC Mastitis Control Program Checklist

1) Establishment of goals for udder health;
2) Maintenance of a clean, dry, comfortable environment;
3) Proper milking procedures;
4) Proper maintenance and use of milking equipment;
5) Good record keeping;
6) Appropriate management of clinical mastitis during lactation;
7) Effective dry cow management;
8) Maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows;
9) Regular monitoring of udder health status;
10) Periodic review of mastitis control program.

Scientific Publication

Somatic Cell Count During and Between Milking

Background & Objectives

Somatic cell count (SCC) is the most frequently used indicator of subclinical mastitis in dairy cattle. Milk samples for SCC analysis as part of Dairy Herd Improvement programs are routinely collected at milking time. For researchers and veterinarians, sample collection during milking may not always be feasible. Furthermore, with increased use of portable somatic cell counters, milk samples are more likely to be taken in between milkings by dairy producers or their advisors.

Diurnal variation was suggested to be the result of proportional dilution relative to milking interval, and is thought to be larger in high producing cows than in low producing cows. With decreasing mean individual cow SCC and increased milk production per cow, it may be possible that the SCC decreases faster post-milking nowadays and samples that are indicative of IMI status can be taken sooner after milking.

The objectives were to determine:

- How sampling time affects the sensitivity and specificity of SCC as an indicator of IMI status?
- Which cells are responsible for the diurnal variation in SCC?

Overview of Methods

- 6 PEI dairy farms were selected that housed their lactating cows in tie-stalls and milked twice daily;
- Each herd was milked AM and PM with a 9 to 10h interval, as measured from the end of AM milking to the start of PM milking;
- Within each herd, 9 to 11 cows were selected that had 4 milk producing udder quarters, no clinical mastitis, and a production of more than 10 kg/day;
- Immediately before AM milking (PRE-AM) and immediately before PM milking (PRE-PM), quarter milk samples were collected;
- Quarter samples half-way through AM milking, immediately after AM milking, and every 60 min. after detachment of the milking unit were collected;
- Sterile quarter milk samples for bacteriological analysis were collected in duplicate at PRE-AM and PRE-PM after SCC samples were taken;
- Samples for differential cell counting were also collected from 20 cows on 2 farms.

Summary of Findings

- Quarter SCC fluctuates during and between milking which has consequences for implementing udder health programs that use individual cow somatic cell count to identify cows with an intramammary infection (Figure 1);
- In quarter samples collected between milkings, SCC is not a reliable indicator of the IMI status;
- Differential cell ratios did not change much during the day in quarters with low SCC, and therefore no specific cell type is attributed to the SCC fluctuation between milkings in these quarters;
- Quarters with an elevated SCC however, showed a relatively higher proportion of polymorphonuclear lymphocytes shortly after milking, followed by gradual decline to pre-milking levels. The proportion of macrophages mirrored this pattern.
Take Home Message

To be able to make optimal interpretations of SCC tests, whether by laboratory, portable SCC devices, or CMT, veterinarians, researchers, and udder health advisors should collect milk samples immediately before milking.

Collecting “Clean” Milk Samples

Strict aseptic procedures must be used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow’s flanks, udder and teats, on the hands of the sampler, and in the barn environment. Briefly, you should:

- Clean udders and especially teats before sample collection;
- Remove and discard a few streams of milk to reduce the number of contaminating bacteria in the teat canal;
- Clean carefully each teat end with a pledge of cotton or gauze sponge moistened with 70% ethyl or isopropyl alcohol;
- Sample the near teats first, then the far ones;
- Do not allow the cap or the vial opening to touch the teat end. A sample volume of 3 to 4 mL is usually adequate;
- Place samples in racks for ease of handling;
- Hold them in an ice container at 5°C before sending them to the laboratory.


Scientific Publication

Effect of Season on Somatic Cell Count and the Incidence of Clinical Mastitis

Background & Objectives

Environmental and climatologic factors affect the incidence of many diseases and disorders in dairy cows, such as mastitis. Therefore, incidence of these diseases often has a seasonal pattern. This seasonal pattern, however, can also be the result of season-specific average stage of lactation of the herd, especially in herds where the calving pattern of dairy cows tends to be seasonal.

Bulk milk somatic cell count (BMSCC), individual cow somatic cell count (ICSCC), and incidence rate of clinical mastitis (IRCM) are all udder health parameters. So far, no studies have been reported on the effect of season on BMSCC, IRCM, and ICSCC in the same herds and time period over multiple years.

The objectives were to determine in the same herds the seasonal pattern over a four-year time period of:

- Bulk milk somatic cell count;
- Elevated individual cow somatic cell count;
- Incidence rate of clinical mastitis;
- Pathogen-specific incidence rate of clinical mastitis.

Overview of Methods

- Based on mean annual BMSCC, three categories of herds were defined;
- For each category, 100 Dutch farms were selected according to specific criteria;
- Information from milk recordings and BMSCC data were provided by the Dutch national milk recording system;
- Farmers were asked to sample cows with signs of clinical mastitis before treatment and record severity of signs, treatment and affected quarter;
- Management data about use of pasture or confinement in summer were derived from a questionnaire conducted on-farm.

Summary of Findings

- Information BMSCC of the 300 farms ranged from 28,000 to 740,000 cells/mL (mean of 187,000 cells/mL);
- Season has an effect on all udder health parameters: BMSCC, individual cow SCC, and IRCM. The amplitude of the seasonal effect on BMSCC differed among the 4 years of the study and peaked in August to September;
- “New” high ICSCC peaked around August while “chronic” ICSCC peaked in the spring;
- Cows were more likely to experience clinical mastitis in late fall (December) than in the summer;
- Effect of season was also clearly present for most pathogen-specific IRCM, except for C. bovis. The peak for most pathogen-specific IRCM was in December or January. S. uberis IRCM and E. coli IRCM in semi and total confined herds peaked in August, October, and June, respectively;
• The increase of BMSCC in August and September cannot fully be explained by IRCM, but is most likely associated with the increase of cows with new high ICSCC and longer periods of high ICSCC;

• Distinguishing between *Strep. uberis*, *Strep. dysgalactiae*, *Strep. agalactiae*, and other streptococci is essential when identifying *Streptococcus* spp., because each of them has a unique epidemiology;

• *Strep. uberis* IRCM seems to be related to pasture, whereas other streptococci and *E. coli* IRCM seems to be more housing-related.

**Take Home Message**

The present study demonstrates the importance of milk culture and differentiation of mastitis pathogens, in order to be able to make specific recommendations in udder health control programs.

**Scientific Publication**

Does Dry Cow Therapy Cause Antibiotic Resistance in Dairy Cows?

Background & Objectives

Antibiotic treatment at drying-off is an indispensable tool used by dairy producers to prevent the occurrence of mastitis prior to calving and to cure persistent udder infections at the end of lactation. However, the development of antibiotic-resistant bacteria is of increasing concern. Consequently, producers and veterinarians often question the potential risk of drug resistance in dairy cattle. Furthermore, consumers are worried about the use of antibiotics in animal production and their potential effect on the environment and on public health.

To date, few studies have dealt with this subject and none have established a clear link between treatment at drying-off and an increase in drug-resistant bacteria. Moreover, the NMC, an international organization dedicated to mastitis reduction, maintains the recommendation to administer treatment to all cows at drying-off to better control mastitis in the herd.

The objective was:

- To determine whether dry cow therapy is associated with the development of antibiotic resistance in bacteria causing mastitis (*Staphylococcus aureus* and coagulase-negative staphylococci) and in bacteria of the intestinal system (*E. coli* and enterococci) over a single dry period.

Overview of Methods

- Eight dairy farms in Quebec and one in Ohio participated in the study. All practiced selective treatment at drying-off;
- Samples of milk and feces were taken before drying-off and after calving, from both treated and untreated cows;
- The bacteria that were targeted and isolated from these samples were subjected to tests regarding their susceptibility to antibiotics.

Summary of Findings

Antibiotic diffusion from mammary gland to blood following treatment

- Analysis of blood plasma of nine cephapirin dry-treated cows and of ten novobiocin/penicillin treated cows confirmed that a small quantity of antibiotic diffuses from the mammary gland to the blood following dry treatment. This diffusion indicates that antibiotics given at dry-off could potentially influence the antibiotic resistance of cows’ intestinal bacteria.

Bacteria that cause mastitis

- When comparing results from treated cows with those of untreated cows, no significant increase of antibiotic resistance could be detected for *Staphylococcus aureus* or for coagulase-negative staphylococci.

Bacteria in the intestinal system

- The *E. coli* of 36% of the treated cows showed an increased resistance to ceftiofur; slightly greater than 28% of non-treated cows.
- With respect to enterococci, there was no evidence to suggest that the treatment at drying-off increases the resistance to the antibiotics tested.
Take Home Message

1. As in previous studies reported in the scientific literature, the results of this study do not show any increase in drug resistance of mastitis bacteria following dry cow therapy. Therefore, because of the effectiveness of this treatment to control mastitis in dairy herds, the recommendation to treat cows at drying-off remains appropriate.

2. The increased resistance of *E. coli* bacteria in the cows’ intestinal systems, on the other hand, does suggest that vigilance by the research community is necessary. Further studies are necessary to corroborate these results and to shed more light on the situation. Although these bacteria, which are excreted in the feces of cows, can end up in the environment and theoretically pose a potential risk to public health, the data do not justify a need for concern at the farm level or for the public’s health.

Proper Use of Medications

- Consult a veterinarian on proper use of all medicines;
- According to label directions, store medicines in a clean, dark cupboard or refrigerator. Check that refrigerator temperature is between 2 – 7 degrees C;
- Do not store medicines with needles in caps;
- Check expiry dates regularly and discard those past the expiry date through your veterinarian. Discard medicines that have changed in appearance (discoloured or thickened);
- Read the product label and OBSERVE WITHDRAWAL TIMES FOR MILK AND MEAT;
- Extra-label drug use should be limited and under the specific advice of the herd veterinarian.


The research team would like to thank the Quebec Department of Agriculture, Fisheries and Food for its financial support, without which this project would not have been possible.
Soluble CD14 and Mastitis Resistance

Background & Objectives

The clinical symptoms of coliform mastitis are primarily due to the cow’s immune response to lipopolysaccharide (LPS), also known as endotoxin. There are two major types of cells in milk that respond to LPS, macrophages and epithelial cells. The former expresses a protein termed CD14 on its cell surface and can secrete it into milk. The protein binds with LPS. The latter cell type does not express CD14, but is stimulated by LPS in the presence of CD14 in milk.

The researchers of this project have previously shown that a high concentration of CD14 has the ability to reduce the severity of coliform mastitis by interfering with the interaction of LPS and epithelial cells. Given those preliminary results, they have hypothesized that dairy cows with higher levels of CD14 in milk are less susceptible to severe clinical coliform mastitis and increasing CD14 in cows may be approached by genetic selection.

The objectives are:

- To characterize the association between CD14 and resistance to clinical coliform mastitis;
- To produce CD14 in transgenic sheep and determine the role of CD14 during coliform mastitis.

Summary of Progress

- Experiments have delineated the mechanism by which CD14 binds with LPS and interacts with mammary epithelial cells. A challenge trial is being planned to demonstrate anti-LPS properties of CD14. Cows that produce low or high levels of CD14 will be experimentally infected with E. coli and the severity of mastitis will be monitored;
- The process to produce transgenic sheep that synthesize high levels of CD14 is on-going. Sheep cells were successfully transfected with the DNA sequence for CD14 and the process is being optimized for the nuclear transfer into donor sheep oocytes.

Potential On-farm Application

CD14 could be used to neutralize LPS during acute E. coli mastitis. Scientists at the United State Department of Agriculture (USDA) have suggested that “the CD14-based product may eventually be commercially developed for use by dairy farmers as a treatment to prevent cows from becoming infected during their dry period”. Our research will provide scientific support for this potential application.

Clean Environment for Clean Cows!

Clean bedding and resting areas will prevent contamination of teat ends from environmental sources and reduce the preparation time prior to milking. Items of interest are good drainage, routine removal of manure, and proper ventilation. Prevent overcrowding in housing areas. Dry cows must be provided the very best of care. Additional areas of concern are freestalls and types of bedding utilized. Pastured cattle must not be allowed to develop wet wallows.

Development and Characterization of a Vaccine Against Coliform Mastitis

Summary of Progress

• A series of microencapsulation techniques were tested with \( E. \ coli \) J5 serving as the vaccine antigen. The researchers were able to optimize the microencapsulation procedure and consistently produced a microcapsule vaccine with maximum loading and an even dispersion of J5 cells within the microcapsule polymer matrix;

• Cows were vaccinated once with various formulation and were observed to have a similar antibody response as that of cows which received a commercially available vaccine that required multiple shots.

Potential On-farm Application

1 - The current results suggest that a single shot of the experimental vaccine can elicit a prolonged antibody response that is comparable to a commercially available, multiple shots product.

2 - The vaccine can eliminate the need for booster shots during the dry period and subsequently reduce labour cost and animal handling. It also has the potential to further increase the immune response of cows, and therefore reduce the severity and duration of clinical coliform mastitis, and subsequent reduction in production loss, treatment, and increase longevity of the cow.

3 - This vaccine can serve as an additional tool to augment existing udder health management procedures to reduce the occurrence of clinical environmental mastitis.

Incidence and Monitoring of New Intramammary Infections

Potential On-farm Application

1 - In large part, the best way to control sub-clinical cases of IMI is to prevent new cases from occurring. Once we have identified risk factors (management practices) associated with a high number of new IMI, we can develop methods of controlling them so as to achieve a long-term reduction in the rate of IMI (Figure 1). By estimating the effect of each of these risk factors on the incidence of IMI, we will also be able to prioritize those actions that will most effectively prevent or solve problems of high rates of IMI according to the various bacteria that cause mastitis.

2 - The new knowledge acquired in this study will guide dairy producers, veterinarians, and dairy-monitoring advisors toward the wider adoption of certain strategies and practices whose value is already recognized. These tools will be based on data that are already routinely available, such as the somatic cell count (SCC) from the cow, the SCC from the bulk tank, the production data compiled by the dairy-monitoring organizations, and bacteriological data.

Background & Objectives

The fundamental objective of any mastitis prevention activity is to reduce the occurrence of new clinical and subclinical intramammary infections (IMI). The IMI incidence rate describes how quickly new IMI are occurring in a herd and is a sensitive measure of how well mastitis control programs are working. Incidence rates also give insight into the dynamics of IMI within a herd and are fundamental data for herd models of IMI. Unfortunately, incidence rates of new IMI are difficult to measure so there is little information about these rates in Canadian dairy herds. Nor do producers have reliable tools for monitoring the incidence of new IMI in their herds. In the future, mastitis researchers and the dairy industry must have benchmarks of mastitis incidence.

The objectives are:
- Developing a scientifically valid definition of subclinical IMI, for use in both lactating and non-lactating cows, using available data such as SCC and/or bacteriological culture results;
- Use the definition of IMI to develop a scientifically valid definition of when a new IMI has occurred within a quarter, based on repeated samplings from the same quarter;
- Determine pathogen-specific incidence rates of new IMI in Canadian dairy herds (cohort herds);
- Evaluate methods which producers can use to estimate new IMI incidence rates in their herds. These methods will be based on routinely available data (e.g. cow-level SCC) and/or reasonably accessible data (e.g. single sample culture results);
- Differentiate risk factors that affect IMI incidence from those that affect IMI prevalence.

Summary of Progress

- Approximately 50% of samples from dry cows and about 65% of samples from lactating cows to determine incidence rates of mastitis have been collected from the National Cohort;
- Results from a series of studies to determine the validity of using frozen milk samples for determination of SCC were positive, even after multiple freeze-thaw cycles. However, the use of the California Mastitis Test on previously frozen milk samples was shown to be an unreliable indicator of mastitis;
- Three questionnaires to capture data about on-farm mastitis monitoring and control practices were developed and are being used in the study of factors that affect the frequency of new and chronic mastitis.

2006-2010
Figure 1. Interrelationships between management practices and udder infections

Integrative Genomic and Proteomic Strategies to Identify Immunological Profiles in Cows Associated with Enhanced Resistance Against Mastitis Pathogens

Background & Objectives

The immune system is composed of genetically regulated sets of cells that control the immune response to pathogens associated with mastitis. The identification of individual cows with high or low immune response characteristics and the underlying genes and proteins that control immunity will allow for the enhancement of mammary gland defense following exposure to mastitis pathogens.

This project explores the genetic regulation of the bovine immune response to mastitis pathogens by:

• Identifying cows with a high or low immune response;

• Determining how the genetic profile of high and low immune responders are associated with resistance or susceptibility to mastitis;

• Identifying novel proteins in resistant and susceptible cows and identifying specific genetic sequences that are associated with the proteins.

Summary of Progress

• Protocols have been developed and are being implemented for the identification of high and low immune responder cows that are enrolled in the National Cohort. Immune response in association with health and production traits is being analyzed. About 500 cows will be tested and hair samples from those cows are being collected as a source of DNA;

• A set of genetic tools used to identify immune response genes of cows has been developed. Preliminary results indicate that different genes are expressed among high and low responder cows, and those genes may be associated with resistance to mastitis;

• Methods are being developed to identify and analyze protein profiles in milk following natural and experimental intramammary infection by various bacterial pathogens. Results thus far indicate that gene expression and the subsequent synthesis of certain proteins by the immune system is specific to the causal pathogen of infections. The development of tests to identify gene sequences that control the immune response, and therefore have an effect on the outcome of mastitis, can be used to identify and possibly select cows with a high degree of resistance toward mastitis.

Potential On-farm Application

Identification of cows with inherently high immune response or mastitis resistance should improve the general health of cows. This will in turn reduce the use of therapeutics with subsequent improvements in food quality and safety, as well as animal well-being.
Identification of Virulence Genes Expressed by Bacterial Pathogens of the Cow Mammary Gland

Background & Objectives

Pathogens such as *Staphylococcus aureus* express specific virulence genes during mastitis. The expression of these genes can lead to the production of proteins that can help it evade the immune defences of the mammary gland to cause chronic mastitis or to produce toxins which cause severe clinical mastitis. The aim of this project is to utilize molecular techniques to identify virulence genes that enable this bacterium to colonise the bovine mammary gland and to cause chronic mastitis.

The objectives are:

- To compare and identify the virulence genes universally found in bacterial isolates that cause chronic mastitis;
- To identify genes that are specifically expressed by *Staph. aureus* in the mammary gland during mastitis.

Summary of Progress

- To date, forty eight *Staph. aureus* mastitis isolates were genetically characterized using DNA microarray technology. The isolates included 11 pairs collected from the same cows 60 days apart, i.e., between dry off and calving. Those isolates were defined as causing chronic mastitis. Mastitis causing isolates were also collected from various farms to help define the genetic diversity of *Staph. aureus* strains within a herd and across farms. Ten of the eleven pairs of isolates that caused chronic mastitis were confirmed as being genetically identical to each other. The majority of these isolates clustered in a particular clonal type and shared identical genetic markers. These molecular markers have the potential to be used as diagnostic tools for the rapid identification of problematic isolates.
- *Staph. aureus* DNA microarrays were also used to identify certain genes that are expressed during experimentally induced mastitis. Such experiments revealed the expression of genes commonly expressed by several isolates and these molecular markers represent excellent candidates for vaccine antigens.

- Results to date suggest that a specific set of virulence genes predisposes some isolates of *Staph. aureus* to cause chronic mastitis and the regulation of their gene expression during mastitis may influence the type of infection – subclinical or clinical mastitis.

Potential On-farm Application

1. The suggestion that specific *Staph. aureus* genes are associated with a propensity to cause chronic infection may drive the development of *Staph. aureus* strain-specific diagnostic assays. This would permit dairymen to target management interventions based on the likelihood of chronic *Staph. aureus* intramammary infection.

2. Identification of certain genes could be useful in developing diagnostic tools for the rapid identification of problematic *Staph. aureus* strains and for identifying antigens for vaccine development.

Early Detection of New Infections

This is necessary to preserve milk flow, insure desirable response to treatment and to prevent chronic infections. Prompt detection of mastitis will preclude severe mastitis outbreaks. Early detection may be done by prestripping prior to milking, observing the udder and teats, the California mastitis test, using various forms of electronic somatic cell counting or electrical conductivity. Multiple detection systems are desirable and should be routinely used. Milkers should be trained to use these techniques and should be aware of the importance of management feedback.

Development of a Nucleic Acid Vaccine Against Staphylococcus aureus Mastitis

Background & Objectives

The complete genomic sequences of Staph. aureus are now available and have lead to the identification of several potential vaccine target proteins associated with virulence factors. The more promising vaccine formulations are based on mixtures of cell surface proteins. These can now be selected from a list which identifies essential proteins for bacterial survival and protection from the host immune system.

A second, but critical, ingredient in a successful vaccine is the combination of adjuvants with the vaccine antigens, which influence the immune response following immunization.

The objectives are:

• To identify a combination of antigens and adjuvants that could be used as a vaccine to protect against Staph. aureus mastitis;
• To evaluate the efficacy of several vaccine formulations in mice;
• To evaluate the immune response and efficacy of optimized vaccine formulations in cows.

Summary of Progress

• Four Staph. aureus recombinant protein antigens were produced and purified, and used to formulate a vaccine that was highly effective in eliciting an antibody response in cows and mice. The enhanced immune response was not only attributed to the purity of the four proteins, but also due to the inclusion of plasmid DNA that acted as a molecular adjuvant in the vaccine;

In order to test the efficacy of the protein antigen-plasmid adjuvant vaccine, mice were immunised with the formulation and then experimentally infected with Staph. aureus. The results clearly showed that mice were protected by the protein-plasmid formulation;

This vaccine formulation is currently being tested in a challenge trial with cows. The trial is on-going and results are not yet available.

Potential On-farm Application

The benefits of a vaccine against Staph. aureus mastitis and Staph. aureus infection are considerable. Although the results to date show great potential for this methodology, they will require considerable development before they are marketable.

Control the Spread of Staphylococcus aureus

Staph. aureus (SA) is a contagious mastitis pathogen that lives primarily on the surface of the skin of the udders and teats of SA-infected cows. It can be transferred from one cow to the next at milking time by contamination of anything that moves from one cow to the next. The major route of spread for SA occurs when liners carrying a small film of milk and SA move from an infected cow to an uninfected cow milked next in the order. SA can transfer from the infected cow’s udder to the teat skin of the new cow.

The use of bactericidal teat-dipping solution has been proven to reduce the incidence (new infection rate) of intramammary infections. For teat dip to prevent the transfer of SA infection, all four teats must be appropriately covered with dip after each and every milking.

Vaccination Strategies to Enhance Mammary Gland Immunity

Background & Objectives

Most *Staph. aureus* vaccines are formulated with traditional adjuvants and delivered by subcutaneous or intramuscular injection at sites far removed from the mammary gland. This practice stimulates immunity in the circulatory system, but is not optimal for local immunity in the mammary gland. Prior to calving, specialized receptors transport antibodies from the circulatory system into colostrum. After calving, expression of the receptors are reduced and the level of antibodies secreted in milk progressively declines to low levels. But, levels increase substantially at the time of mammary gland involution following dry-off. Researchers of this project aim to develop improved methods of enhancing immune defences in the mammary gland against *Staph. aureus* mastitis.

Objectives include:

- Optimizing the magnitude of immune response;
- Optimizing local mammary gland immunity;
- Testing a nucleic acid vaccine.

Summary of Progress

- Previous work demonstrated that a vaccine formulated with proteins from the cell wall of *Staph. aureus* (GapC/B proteins) induced a significant increase in antibody production in mice. Cows were vaccinated with the GapC/B proteins and exhibited an excellent immune response to the antigens;
- The GapC/B protein antigen was also used to investigate optimization of local mammary gland immunity. Although immunization increased antibody levels, vaccine administration in an area close to the mammary gland did not appreciably enhance mammary gland antibody levels when compared to administering the vaccine in the neck area;
- A plasmid DNA vaccine encoding the gapC/B gene was compared to the GapC/B protein vaccine. Results indicated that immunization with the plasmid DNA vaccine did not significantly increase serum antibody concentration relative to immunization with the conventional GapC/B protein-based formulation.

Potential On-farm Application

1. The enhancement of immune responses through the use of novel vaccine formulations will ultimately translate into a longer duration of immunity, reduced frequency of immunization and a safer product due to reduced antigen load.
2. Furthermore, the results are broadly applicable to other vaccines currently used.
Validation of On-farm Mastitis Pathogen Identification Systems and Determination of the Utility of a Decision Model to Target Therapy of Clinical Mastitis During Lactation

Background & Objectives

Mastitis cases that are caused by gram positive infections respond more favourably to treatment. In Canada, some of the antibiotic used to treat clinical mastitis may not be justified because of poor efficacy of treatment against certain pathogens such as E. coli. Small-scale studies have been conducted to find appropriate tools to determine, in a timely manner, whether antibiotic therapy is justified in the treatment of clinical mastitis. This project tests the hypothesis that use of tools that rapidly identify mastitis pathogens will enable sound therapy decisions and thereby reduce the ineffective use of antibiotics to treat mastitis.

This study is divided into two phases:

• The first phase examined on-farm culture media tools for the rapid identification of bacteria in milk samples collected from farms in the Atlantic region and in regions of Quebec;

• The second phase examines a tool selected from the first phase on a larger scale. The rapid identification tool will be tested to determine its ability to impact treatment strategies and treatment success on all participating farms enrolled in the National Cohort.

Data generated from the second phase will allow for the formulation of rules for the treatment of mastitis and for monitoring effects on farm antibiotic use, disease outcome, mastitis cure rates and overall incidence of clinical mastitis on the farm.

Summary of Progress

• The ability to identify bacterial groups appropriate for making a treatment decision (Gram positive (treatment), Gram negative and No Growth (no treatment)) was very good;

• When considering a targeted treatment program, two measures are most important. First, the proportion of the animals truly needing treatment (Gram positive) that are identified (sensitivity) and secondly, the proportion of the animals which were not treated that would not have benefited from treatment (negative predictive value);

• Using the combination of Petrifilm™ Total Aerobic Count and Coliform Count yielded a sensitivity and negative predictive value of >90%;

• Using Biplates yielded a sensitivity and negative predictive value of >90%;

• The agreement between the five readers was very high for both testing systems;

• Results indicate that both systems give acceptable results for making an on-farm treatment decision, however, due to on-farm logistics (mainly shelf-life) the Petrifilm system was selected for on-farm application.

• Testing of tools in cooperating NCDF herds is being conducted. Once sufficient data has been acquired, treatment rules will be developed and tested to monitor effects on farm antibiotic use, disease outcome, and overall incidence of clinical mastitis on the farm.
Potential On-farm Application

A commercially available on-farm culture system will play an important role in a mastitis treatment protocol. It will enable producers to make decisions based on results and ultimately contribute to the reduction in the use of antibiotics on farm, while maximizing cow health and welfare outcomes.

Reasons for Pretreatment Negative Culture Results

Reports indicate that 25 to 40 percent of all clinical samples are negative on routine culturing (no growth). Reasons include:

- Numbers of certain organisms, such as mycoplasma, *Staphylococcus aureus*, and coliforms, can vary greatly in infected quarters, and may occasionally be less than the minimum detection limit of the assay. The minimum detection limit when plating 0.01 mL of milk is about 100 colony forming units per mL;
- The organism may no longer be present and the clinical signs are due to by-products such as endotoxins;
- Somatic cells may have phagocytized (destroyed) the organisms;
- The organism may require cultural conditions other than those used for isolation (i.e. reduced temperature, prolonged incubation, special media, anaerobic conditions (without oxygen), etc.).

Association Between Antimicrobial Use in Mastitis Treatment and Antimicrobial Resistance

Background & Objectives

In Canada, mastitis treatment accounts for more than half of all antibiotics used by dairy producers. There is much concern regarding the potential for the development of antimicrobial resistance in bacteria as a result of antibiotic use in agriculture. The general assumption is that the rate of antimicrobial resistance is closely associated with the rate of antimicrobial use. However, this assumption has yet to be demonstrated in a dairy environment. Currently, conclusive information on antimicrobial use in Canadian dairy cows and the degree of antimicrobial resistance in mastitis pathogens is not available.

The objectives are to:

- Determine antimicrobial use in Canadian dairy herds;
- Determine antimicrobial resistance of mastitis pathogens isolated from Canadian dairy herds;
- Describe changes in occurrence of resistant pathogens and its association with the antimicrobial use on dairy herds. Dairy farms that are enrolled in the National Cohort of Dairy Farms (NCDF) are monitored for two years to fulfill the objectives.

Summary of Progress

- Information on antimicrobial use on dairy farms is being collected in conjunction with data collection from the NCDF project. To date, data recording sheets have been distributed to all NCDF coordination centers and waste receptacles for the disposal and subsequent collection of antimicrobial product are in place on participating farms. Receptacles are being collected monthly and the contents recorded. Information from this first year of data collection is currently being compiled;

- Approximately 700 bacterial isolates from the Mastitis Pathogen Culture Collection have been selected and are undergoing antimicrobial resistance testing. At least 2,000 Staphylococcus aureus isolates and 1,000 Escherichia coli isolates will be evaluated for antimicrobial resistance by the end of this study;

- Once sufficient information is available, data analysis will commence to determine the association between antimicrobial use on individual farms and antimicrobial resistance patterns of mastitis pathogens recovered from quarter milk samples from cows on those farms.

Potential On-farm Application

1. The drugs which are most commonly used in Canadian dairy farms for mastitis cure and prevention will be known and the development of resistance in udder pathogens against these drugs will be determined;

2. In case of a significant positive association between use of such drugs and development of AMR, judicious use of antibiotics on farm will become more and more important. For example, if a particular antibiotic fails to cure mastitis but is still being used, it can become a potential source of developing AMR and hence some alternative drug can be considered.
Resistance 101

What is it?
Antibiotic resistance is the ability of bacteria to grow and multiply in the presence of an antibiotic. Resistance develops in bacteria with genes enabling them to produce proteins that protect against the effects of the antibiotic molecule.

Is it reversible?
In theory, bacterial populations that have become resistant to a certain class of antibiotics can become susceptible to them again after a prolonged discontinuation of antibiotic use.

How does resistance develop?
1. By selection pressure. Bacterial populations often contain some cells susceptible to a given antibiotic and others more or less resistant. If an antibiotic administered at a low dose kills susceptible cells but fails to kill moderately resistant bacteria, the surviving bacteria will remain in the mammary gland, free to multiply and become the dominant strain. The bacterial population would then become more resistant.

2. By gene transfer. A number of the bacterial genes associated with antibiotic resistance are carried by plasmids, small DNA molecules found in a variety of bacterial species. Plasmids can be transferred between bacterial cells. When plasmids containing antibiotic-resistant genes are transferred to other bacteria, the recipient bacteria acquire resistance to antibiotics.

3. By induction. Bacterial genes must be turned on or induced to express the proteins required for antibiotic resistance. Factors that induce antibiotic resistance are not well understood, and are the focus of several research projects, especially in human medicine.

Knowledge Transfer Activities

The sharing of knowledge and technologies with users – producers, veterinarians, agronomists, technical advisers, institutions, and other members of the Canadian dairy sector – is a priority for the CBMRN. Our major source and heart of information dissemination is the “Mastitis: Online Resources” section on our website. Under this heading, readers can find various documents about the monitoring and control of mastitis: PowerPoint, PDF files from dairy magazines, transfer sheets, and NMC publications. The majority of documents are available in both English and French for our domestic and international users. The website can be accessed in English (www.mastitisnetwork.org) or in French (www.reseaumammite.org).

Our knowledge diffusion activities exploit different media and methods of communication, and utilize several channels that are already in use by the dairy community. A Mastitis Column is published regularly in “The Milk Producer” and in Le Producteur de lait québécois where mastitis research results are presented. The monthly newsletter “Mastitis-flash” and the “CBMRN Bulletin” are sent to our subscribers using an electronic mailing list. Have you registered for a subscription? If not, visit our website today and fill out the form!

For each of the projects presented in this booklet, a transfer sheet is available on our website under the “Research Results” heading in the “Mastitis: Online Resources” section. These transfer sheets will be up-dated yearly for the benefit of our users. We invite you to take a few minutes out of your busy schedule to explore our website where useful information is readily available for your client or yourself. Cultivating knowledge to maintain milk quality is not only a catchy slogan but a goal of the CBMRN in controlling mastitis to benefit everyone involved in the dairy industry.
The “International Directory of Mastitis Researchers” gathers the principal mastitis researchers in the world. This highly visual directory is presented in a database format. The search tools include menus and options of classification allowing a quick and simple way to access the information.

To restrict the size of the directory, seventeen countries representing four continents were recognized as being the most active in mastitis research. For each country, only researchers who are the principal author or one of the co-authors of scientific articles published in English, between 2003 and 2008, in a national or international renowned publication were selected. We have prioritized about twenty five researchers per country who published the greatest number of mastitis related publications during this time span. For certain countries where numerous mastitis research projects are being conducted, such as the USA, Canada, or Germany, for example, a minimum limit of two or three articles has been set to be listed in the directory. Thus, for those countries, the database actually contains information on thirty to sixty researchers. The nationality of researchers has been established according to their current workplace and not according to their countries of origin.

Information about researchers is classified into two main themes according to research interest:

(1) Mastitis control – which includes management, milking procedures and equipment, therapy, and immunity categories;

(2) Mastitis detection and diagnosis – which includes somatic cell count, milk components, bacteriology and pathogens categories.

Scientists can also be cross referenced by the particular pathogen associated with their research.

The main sources of information for research publications were the following:

• ISI Web of Knowledge-WEB of SCIENCE (http://apps.isknowledge.com);
• PUBMED (http://www.pubmedcentral.nih.gov);
• INIST-Institute of Scientific and Technical Information - Centre National de la Recherche Scientifique (CNRS) of France (http://www.inist.fr).

This tool will promote CBMRN collaboration with the international scientific community and will support the creation of global links. The International Directory of Mastitis Researchers is available in the “Mastitis Around the World” section of the CBMRN website.

**Mastitis Around the World**

“Mastitis Around the World”, found under the “Mastitis: Online Resources” section of our website, presents main activities related to mastitis in the different region of the world. By clicking on a region of a map, surfers can access five different pages that represent each continent of the world. Within each continent, a list of major countries involved in mastitis research or have an interest in udder health activities can be reviewed. For each country, dairy statistics and a list of principal research groups or institutions may be available. For each group, an “In brief” section gives a description of “Who are they?” and “What do they do?” Links with their latest publications and website, if available, is also included on the “In brief” page. Here is a mastitis world to discover! Have a nice trip!
How to Contact Us?

Canadian Bovine Mastitis Research Network
Faculté de médecine vétérinaire
Université de Montréal
C.P. 5000, Saint-Hyacinthe (Québec) Canada J2S 7C6
Phone: (450) 773-8521, ext. 8619
Fax: (450) 773-8179
Website: www.mastitisnetwork.org

Administrative Team

Daniel Scholl
Scientific Director and Coordinator of the Core Research Platform
Phone: (450) 773-8521, ext. 8605
Daniel.scholl@umontreal.ca

Grant Tomita
Scientific Assistant
Phone: (450) 773-8521, ext. 8621
Grant.tomita@umontreal.ca

Annik L’Espérance
Network Manager
Phone: (450) 773-8521, ext. 8619
Annik.lesperance@umontreal.ca

Julie Baillargeon
Transfer Manager
Phone: (450) 773-8521, ext. 8620
Julie.baillargeon@umontreal.ca

Hélène Poirier
Information Agent
Phone: (450) 773-8521, ext. 0066
Helene.poirier@umontreal.ca

Photographs
Kristen Reyher p. 2
Istockphoto – cover and p. 10, 11, 21, 23, 27
Laboratoire de bactériologie clinique – FMV, Saint-Hyacinthe – p. 25
Jean-Yves Perrault p. 23

Graphic Designer
Tommy Ferland, La Fabrik

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