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We are very happy to present you with the Canadian Bovine Mastitis Research Network’s (CBMRN) second Annual Report. The year 2007-2008 was a year of advancing to a stage of maturity after our first year of laying foundations.

Progress was no where more evident than at the 2007 Annual Scientific Meeting in November in Montréal. A record number of participants were involved in presentations and posters, workshops and discussions, and exchanging of ideas. Networking within CBMRN has come into its own.

In this report you will see that the CBMRN research program is advancing, bringing answers to scientific questions that impact on our understanding of bovine mastitis and how to monitor and control it. The research program is brought to fruition while maintaining a close connection with Canadian dairy producers through their broad participation in the National Cohort for Dairy Farms.

Research is not the only domain where CBMRN saw progress this last year. As research results begin to emerge, our knowledge transfer activities are shifting away from communicating existing mastitis knowledge to dairy producers and advisors toward communicating new knowledge in the context of the existing knowledge. We’ve been given a boost in this undertaking with a supplementary Strategic Network Enhancement Initiative grant from our NSERC partner.

We are grateful to the entire CBMRN team — the researchers, trainees, partners, committee and administrative members — for making these great accomplishments possible. Together, as a team, we can fulfill our objectives and goals for this new coming year of research, training and technology transfer.

Pierre Lampron  
Steering Committee Chairman

Daniel Scholl  
CBMRN Scientific Director
2007-2008
Steering Committee

James Bellamy (non-voting member)
Associate Dean of Graduate Studies and Research, Faculty of Veterinary Medicine, University of Prince Edward Island

James Cross
Associate Dean, Research and Graduate Education, Faculty of Veterinary Medicine, University of Calgary

Annik L’Espérance (March to November 2007) (secretary, non-voting member)
Eliane Auger (October 2007 to March 2008) (secretary, non-voting member)
CBMRN Network Manager

Pierre-Richard Gaudreault
Director, Service de la recherche et de la création, Université de Sherbrooke

Michael Hall (March to October 2007)
Reint-Jan Dykster
(November 2007 to March 2008)
Dairy Farmers of Canada

Mario Jacques (non-voting member)
Associate Dean, Research and Graduate Studies, Faculté de médecine vétérinaire, Université de Montréal

Eileen Jessop (non-voting member)
Portfolio Manager, NSERC

Pierre Lampron, Chairman
Dairy Producer, Fédération des producteurs de lait du Québec

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Conseiller en gestion de la recherche, Université Laval

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CBMRN Scientist, Vaccine and Infectious Disease Organization, University of Saskatchewan

Jim Richards
External Expert, National Research Council Canada, Institute for Biological Sciences

Danielle Rivard
General Manager, Novalait inc.

Guillaume Roy
Research Officer, Université de Montréal

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CBMRN Scientific Director, Université de Montréal

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Science Director, Livestock Production Systems, Agriculture and Agri-Food Canada

Bill Swan
Dairy Producer, Dairy Farmers of Manitoba

Ron Versteeg
Dairy Producer, Dairy Farmers of Ontario

Alan Wildeman
Vice-President (Research), University of Guelph

2007-2008
Scientific Committee

Julie Baillargeon (March to October 2007) (non-voting member)
Anne-Marie Christen (November 2007 to March 2008) (non-voting member)
CBMRN Transfer Manager

Herman Barkema
CBMRN Scientist and Monitoring Theme Leader, University of Calgary

Bruce Beattie
(March 2007 to January 2008)
Wim Ruysch (February to March 2008)
Dairy Producer, Alberta Milk

Ron Maynard (March to October 2007)
Reint-Jan Dykstra
(November 2007 to March 2008)
Dairy Producer, Dairy Farmers of Canada

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Coordonnatrice de valorisation, Novalait inc.

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Portfolio Manager, NSERC

David Kelton
CBMRN Scientist, University of Guelph

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Annik L’Espérance (March to November 2007) (secretary, non-voting member)
Eliane Auger (October 2007 to March 2008) (secretary, non-voting member)
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Andrew Potter
CBMRN Scientist, Vaccine and Infectious Disease Organization, University of Saskatchewan

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Daniel Scholl
CBMRN Scientific Director, Université de Montréal

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CBMRN Scientific Assistant, Université de Montréal

Ron Versteeg
Dairy Producer, Dairy Farmers of Ontario

Xin Zhao
CBMRN Scientist, McGill University

2007-2008
Transfer Committee

Pauline Bilodeau
Technology transfer officer, Agriculture and Agri-food Canada

Vincent Caldwell
Dairy Practitioner, Clinique vétérinaire Coaticook

Julie Baillargeon (March to October 2007)
Anne-Marie Christen (November 2007 to March 2008)
CBMRN Transfer Manager

Kara Irving
Dairy Farmer, Dairy Farmers of Nova Scotia

Steve Mason
Chief Editor, Dairyman’s Digest

Daniel Scholl
CBMRN Scientific Director, Université de Montréal

Guy Séguin
Udder Health Specialist, Dairy Farmers of Ontario

Étienne Tremblay
Advisor, Valacta

2007-2008
Scientific Advisory Panel

Theo Lam
GD, The Netherlands

Scott McDougall
Animal Health Centre, New Zealand

Larry Smith
Ohio State University, USA
MEMBERS, COLLABORATORS, AND FUNDING PARTNERS

2007-2008 ACTIVE MEMBERS

Herman Barkema
University of Calgary

Émile Bouchard
Université de Montréal

John Campbell
University of Saskatchewan

Luc DesCôteaux
Université de Montréal

Trevor DeVries
University of Guelph

Ian Dohoo
University of Prince Edward Island

Philip Griebel
VIDO – University of Saskatchewan

Neil A. Karrow
University of Guelph

Greg Keefe
University of Prince Edward Island

David Kelton
University of Guelph

Pierre Lacasse
DSRDC – Agriculture and Agri-Food Canada

Kenneth Leslie
University of Guelph

Bonnie Mallard
University of Guelph

François Malouin
Université de Sherbrooke

J T. McClure
University of Prince Edward Island

Serge Messier
Université de Montréal

Jose Perez-Casal
VIDO – University of Saskatchewan

Andrew Potter
VIDO – University of Saskatchewan

Claude Robert
Université Laval

Jean-Philippe Roy
Université de Montréal

Daniel Scholl
Université de Montréal

Moussa Sory Diarra
PARC – Agriculture and Agri-Food Canada

Henrik Stryhn
University of Prince Edward Island

Brian Talbot
Université de Sherbrooke

Grant Tomita
Université de Montréal

John VanLeeuwen
University of Prince Edward Island

Xin Zhao
McGill University

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University of Prince Edward Island

Patrick Boerlin
University of Guelph

Vilceu Bordignon
McGill University

David Léger
Public Health Agency of Canada

Paula Menzies
University of Guelph

John Middleton
University of Missouri

Musangu Ngeleka
University of Saskatchewan
### 2007 – 2008 Funding Partners

| Agriculture and Agri-Food Canada | Natural Sciences and Engineering Research Council |
| Alberta Milk                   | Novalait inc.                                  |
| Canadian Dairy Network         | Public Health Agency of Canada                 |
| Dairy Farmers of Canada        | Technology PEI Inc.                            |
| Dairy Farmers of New Brunswick | Université de Montréal                         |
| Dairy Farmers of Nova Scotia   | University of Prince Edward Island             |
| Dairy Farmers of Ontario       |                                                |
| Dairy Farmers of Prince Edward Island |                                            |

### 2007 – 2008 Donors and Sponsors

- CanWest DHI
- Pfizer Animal Health
- Valacta

A number of individuals and organizations are essential to the success of this Network. We would like to thank our funding and research partners, donors, and sponsors for their support, cooperation, and confidence. We would also like to thank the members of the CBMRN committees for their time, commitment, and guidance. Finally, this Network would not be possible without the tireless dedication of all its members, collaborators, students, and technicians.
THE CBMRN MISSION
AND FUNDAMENTAL OBJECTIVES

The mission of the CBMRN is to mobilize national and international scientific and financial resources to decrease the incidence of mastitis, reduce financial losses, and maintain milk quality through concerted research, and effective and rapid transfer of results to end-users.

Fundamental Objectives:

1. To define the mastitis problem on a Canadian level in order to focus long-term research, training, and transfer programs.
2. To maintain excellence in networking, training, transfer, and program evaluation.
3. To conduct research to elucidate new mastitis solutions with near-term transferability.
4. To transfer new mastitis knowledge and technology to dairy producers.
5. To establish a foundation for long-term network research and transfer activities.
NETWORKING AND TRAINING HIGHLIGHTS

• 34 active members and collaborators.
• 10 research partners and 14 funding partners.
• $250,000 from private partners; 8% of the total budget for Year Two funding.
• Additional funding of $200,000 from NSERC (Strategic Network Enhancement Initiative (SNEI)).
• An electronic mailing list of nearly 800 people.
• 21 graduate students, 8 postdoctoral fellows, 28 undergraduate students and 23 technicians.
• A total of 13 within Network collaborators and 23 external collaborators.
• The 2007 Annual Scientific Meeting in Montréal; over 90 scientists, students, technicians, and partners attended.
RESEARCH HIGHLIGHTS

- The Core Research Platform is fully operational. Ninety one farms were recruited into the National Cohort of Dairy Farms and the first round of data collection activities is complete. Milk samples are being analyzed by the National Mastitis Laboratory Network, bacterial isolates are being submitted to Mastitis Pathogen Culture Collection, and isolates are being distributed to researchers.

- Mathematical models that are based on a variety of udder health information sources are being formulated and validated to clearly describe the epidemiology of existing and new intramammary infection.

- Molecular methods have been developed to characterize *Staphylococcus aureus* virulence genes that may predispose some strains of *S. aureus* to cause chronic mastitis.

- Genetic and protein indicators of bovine resistance or susceptibility to mastitis are being identified.

- Cells have been transfected with genes needed to transgenetically produce specific proteins to combat clinical coliform mastitis.

- Prototypes of protein and DNA *S. aureus* vaccines, and a microencapsulated *Escherichia coli J5* vaccine have been developed. Trials are being planned or are currently underway to test the efficacy of the various vaccine formulations.

- Two methods for the rapid identification of mastitis pathogens in milk samples were found to be suitable for on-farm use. Extensive testing in conjunction with the National Cohort of Dairy Farms is being conducted to validate rapid identification methods.

- Data on dairy farm antibiotic use is being collected in conjunction with the National Cohort of Dairy Farms project and bacteria from the Mastitis Pathogen Culture Collection are being tested for antimicrobial resistance.
**THE IMPACT OF THE CBMRN**

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. For its part, the CBMRN fulfills its mission by mobilizing national and international scientific and financial resources to conduct concerted research, and to effectively and rapidly transfer knowledge and technology. Accomplishments in addressing the CBMRN mission are evaluated through five impact areas: 1) research that is actionable and accessible at the farm-level, 2) developmental research, 3) the transfer of existing and new knowledge and technology, 4) the building of capacity for research and transfer, and 5) the National Cohort of Dairy Farms.

<table>
<thead>
<tr>
<th>Impact Area</th>
<th>Expected Outcomes</th>
<th>Targets for Year 5</th>
</tr>
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<tbody>
<tr>
<td>Research that is actionable and accessible at the farm-level</td>
<td>• Knowledge from applied research results transferred to the Canadian industry</td>
<td>• Completion of all research projects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Transfer of all completed projects to the producers</td>
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<tr>
<td></td>
<td></td>
<td>• 2 or more new projects</td>
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<td></td>
<td>• 1 or more new funding source</td>
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<td></td>
<td></td>
<td>• At least 20 international publications and presentations</td>
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<tr>
<td>Developmental research</td>
<td>• Technological research results transferred to the animal health industry for commercial development</td>
<td>• Evolution of the CBMRN knowledge transfer program</td>
</tr>
<tr>
<td></td>
<td>• Fundamental research results leading to follow-up studies that are directed toward transferable technology</td>
<td>• Availability of high quantity of information for the Canadian industry; at least 200 mastitis related articles</td>
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<tr>
<td></td>
<td></td>
<td>• Utilisation of transfer strategies by about 50% of veterinarians and advisors</td>
</tr>
<tr>
<td>Transfer of existing and new knowledge and technology</td>
<td>• Uniform access by Canadian dairy producers and allied professionals to objective, credible, up-to-date, and bilingual information for mastitis control and monitoring</td>
<td>• Efficient transfer program</td>
</tr>
<tr>
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<td>• 50 or more highly trained personnel within the CBMRN (students, post-doctoral fellows, and technicians)</td>
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<td></td>
<td></td>
<td>• 50 or more domestic and international researchers collaborating in mastitis research through the CBMRN</td>
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<td></td>
<td>• Establishment and standardisation of at least one new milk bacteriology laboratory</td>
</tr>
<tr>
<td>Building of capacity for research and transfer</td>
<td>• Newly trained researchers, technicians, and advisors</td>
<td>• Decreased in bulk tank somatic cell count by at least 10% by 2011</td>
</tr>
<tr>
<td></td>
<td>• Bacteriology laboratories with new capabilities to carry out milk bacteriology analyses for diagnostic or research purposes</td>
<td>• On-farm adoption of two or more udder health monitoring or control technology by 2011</td>
</tr>
<tr>
<td></td>
<td>• Existing domestic and international researchers contributing to new Canadian mastitis research projects</td>
<td></td>
</tr>
<tr>
<td>National Cohort of Dairy Farms</td>
<td>• Producers in direct contact with CBMRN research, researchers, and technicians will promote implementation of mastitis management practices</td>
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</tr>
<tr>
<td></td>
<td>• Reduction in bulk tank somatic cell counts</td>
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</tbody>
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**Impact indicators**

**Where do we stand?** Indicators of the CBMRN’s impact have been compiled during the course of the year and are now presented in their appropriate section within this annual report.
The CBMRN has ten research partners and fourteen funding partners, mainly dairy producers’ organizations. For the year 2007 – 2008, private partners of the CBMRN contributed $250,000 to research activities, which accounted for 8% of the total budget. Over the year these partners also contributed their time to the management and direction of CBMRN as members of the Steering Committee, the Scientific Committee, the Transfer Committee and by participating in the Partners’ Annual Review and the Industry Workshop at the Annual Scientific Meeting. In the upcoming year, the CBMRN will actively seek affiliations and partnerships with commercial organisations to promote the transfer of technology into stakeholders’ hands. Such partnerships are key to our success.

Research Partnerships

The CBMRN’s research partnership with Maritime Quality Milk (MQM), which was established in 2006 - 2007 continues to bear fruit. This partnership facilitates the CBMRN’s research realisations in Prince Edward Island, New Brunswick and Nova Scotia and it contributes to MQM’s ability to focus current Canadian mastitis research activities and results on dairy farms in these provinces. The partnership enables CBMRN scientists at the Atlantic Veterinary College to expand their research within the CBMRN scientific program. Funding for MQM was facilitated in part by the CBMRN program because it assures a critical mass of activities, trainees, and research funding at the Atlantic Veterinary College. For its part, MQM provides the CBMRN with an opportunity to share professional and technical human resources when both programs’ needs are for part-time personnel only. The Maritime regional laboratory of the Mastitis Laboratory Network will function within the MQM organization. The Network’s impact on facilitating the establishment of MQM is a big step toward realizing the CBMRN’s mission of providing cooperative mastitis research, training, and transfer to stakeholders.

One of the CBMRN strengths is the ability to encourage research that compliments its own scientific program. During 2007-2008, two new research projects, one representing a new collaboration, were formalised by the CBMRN Scientific Committee. Neither of these projects would have been possible without a resource like the CBMRN Core Research Program and both accentuate the impact of the Platform on mastitis research. During 2008-2009, the CBMRN will actively pursue additional international collaborations, making use of the Strategic Network Enhancement Initiative grant recently awarded by NSERC.
In the knowledge transfer domain, the CBMRN pursued its mission as a true source of credible and objective information for the Canadian dairy industry. The sharing of knowledge and technologies with users – producers, veterinarians, agronomists, technical advisers, institutions and other members of the Canadian dairy sector – remains a priority for the new Transfer Committee formed in June 2007. The Committee members agreed on a transfer program that stresses language translation, communication with users, website development, and dissemination tool building as the main priorities for the coming year. A program was developed to identify performance indicators that measure the impact of the knowledge transfer program on the Canadian dairy industry.

The Knowledge Transfer Program obtained additional funding from a new NSERC funding program called the Strategic Network Enhancement Initiative (SNEI). In 2007 – 2008, the Program received $99,600 and will receive an additional $104,901 for 2008 - 2009. Welcomed with enthusiasm, this supplementary funding serves to strengthen student training, develop and enhance international network collaboration, and augment knowledge transfer activities.

This new initiative also allowed the CBMRN to hire additional personnel. Hélène Poirier joined the administrative team in December with a mandate to define international research in mastitis and related activities. She is also responsible for the organization of workshops to train veterinarians to effectively provide information on mastitis prevention and control directly to Canadian dairy farms. The workshops will be modeled on those offered by the UGCN, the Dutch Udder Health Centre. With this training, Canadian veterinarians will be able to communicate with dairy producers in an interactive way and dynamically deliver information on various topics related to mastitis.

Knowledge diffusion

The main objective of knowledge diffusion is to provide useful and practical information to users: such as to inform them of the best practices for udder health management and to communicate current research developments. Knowledge diffusion activities exploit different media and methods of communication, and utilize several channels that are already in place and in use by the dairy community.

Mastitis: Online Resources

During the last year, the heart of our website, “Mastitis: Online Resources”, kept improving with the addition of various documents: PowerPoint, PDF files from dairy magazines, Transfer sheets, and NMC publications. To grab the web surfer’s interest, attractive pictorial designs were created for articles posted on the web. We also ensured that the majority of documents are available in both English and French for our domestic and international users: primarily dairy producers, veterinarians and professionals of the dairy community. The website can be accessed in French (www.reseaumammite.org) or English (www.mastitisnetwork.org).
In 2007-2008, an average of 489 visitors per month visited our website and viewed 38,388 pages. Seventy one percent of the pages were viewed by Canadian users.

Placed on our website in the fall 2007, the Mastitis Library provides viewers with a selection of books and reference articles on mastitis. To call the attention of event coordinators, and to better identify potential speakers on the topic of mastitis, we have created a new heading called “PowerPoint” under the Mastitis: Online Resources page heading. All electronic mastitis presentations gathered by the Network are laid out in the link with the name of speakers and the associated program. A calendar of events is also provided in the Mastitis: Online Resources section of our website for those who want to find out when an event or conference on udder health is scheduled throughout the world.

A communication plan was formulated to ensure that our project results and recommendations reach the appropriate target users by means of different modes of communication. In the section entitled “Research Results”, three transfer sheets were posted last year:

- Effect of season on the somatic cell count and incidence of clinical mastitis;
- Somatic cell count during and between milkings;
- Evaluation of mastitis pathogen identification for on-farm application.

In the coming year, emphasis will be placed on useful management tools and protocols for dairy producers and their advisers in the Toolbox section of the website. Many tools exist, but are not easily accessible or available in both English and French. A section entitled “Pathogens” will also be launched in the near future. This section will describe microorganisms that cause bovine mastitis. It will include a general description of the pathogen, mastitis symptoms they cause, pictures, and related literature.

Mastitis Column

The Mastitis Column is used to present CBMRN research and results in a practical and useful context for dairy producers. The Mastitis Column is published in two dairy magazines; “Le Producteur de lait québécois” (4 articles in French) and “The Milk Producer” (3 articles in English). The former publication is distributed in the province of Québec (10,124 copies in circulation) and the latter is distributed in Ontario, Maritimes provinces, Manitoba, Saskatchewan, and British Columbia (10,500 copies in circulation). In both publications, the majority of the readership is dairy producers, but the magazine is also distributed to professionals in the dairy sector. The Mastitis Column is also published in an electronic version on our website and on the Agri-Réseau website (Québec). Participants of the National Cohort of Dairy Farms also receive copies on a regular basis.
Mastitis-flash

In November, we launched a new communication tool called "Mastitis-flash". The Mastitis-flash is a monthly English and French electronic newsletter sent to users through our electronic mailing list. It consists of a short list of topics with a brief description to perk the interest of the reader, and contains links leading to full articles on our website. The goal of this newsletter is to help the reader explore our website and to encourage repeat visits.

Frequently Asked Questions (FAQ)

The goal of this activity is to provide answers to mastitis questions from producers. We have accumulated a list of questions and topics which producers can choose and get a response from an expert. These questions are general and non-specific and are addressed in a short article format. Those articles are published in the "Frequently Asked Questions (FAQ)" section of our website. The articles are also sent to regional agricultural publications throughout Canada (in French and English) and to dairy producer associations for publication in their monthly newsletter. Website surfers are invited to send in their questions to the Transfer Manager.

Others Publications

We are required at times to collaborate on articles in other publications. For example, we contributed to an article entitled "Portrait de la mammite clinique au Canada" for the Dairy Farmers of Canada (DFC) Action-Fax in October 2007, and for a brief summary of the Networks’ activities in the DFC ACTION + in Fall 2007. Other collaborations are listed in the Publications and Conferences section of this report.

Knowledge Diffusion Events and Activities

In the past year, the CBMRN participated in numerous dairy related events throughout the world: Heifer Mastitis Conference (June 2007, Ghent, Belgium), Mastitis Research Workers Conference (November 2007, Minneapolis, United States), Colloque sur la santé des troupeaux laitiers (December 2007, Québec, Canada), NMC 47th Annual Meeting (January 2008, New Orleans, United States), and Dairy Focus (March 2008, Nova Scotia, Canada). These were excellent opportunities for Network members to meet current and future users of our Transfer Program, and to form links with different organizations throughout the world. Also, these events increased worldwide exposure of CBMRN research and the communication of useful information.

In two events – the 2008 Dairy Research Communication & Extension Event held in February at the University of Guelph and the Western Canadian Dairy Seminar held in March 2008 in Red Deer, Alberta - a total of 110 packets with pertinent Network information (transfer sheets, CBMRN Bulletin, website information, CBMRN brochure) were distributed to participants.

The CBMRN has a free-standing display on which mission statements and an outline of CBMRN activities can be exhibited during conferences and colloquiums. Various documents such as the CBMRN brochure, Mastitis Column, Mastitis-flash, and NMC publications can be posted on the display for distribution to conference attendees. People at the display are invited to register to be placed on our electronic mailing list. The layout of the display allows for a continuous-loop PowerPoint presentation that can be used to introduce the Network's activities.

The CBMRN is a member of the organizing committee of the "Colloque sur la santé des troupeaux laitiers" and took part in developing the 2007 conference. Participation in this committee enables us to ensure that the subject of mastitis is included in the conference program and that the Québec producers and veterinarians
have access to cutting-edge information in French. A list of CBMRN speakers and subjects is available to organizers of Canadian dairy events and will ensure that results from the CBMRN research program will appear in scientific conferences for the coming years.

A new activity offers CBMRN mastitis documents and information (website information, technical articles, and mastitis columns) to dairy production teachers for distribution to the next generation of producers and advisors. Thus far, sixty information packets were distributed to two agricultural schools and this activity will surely increase over the coming year.

Finally, the CBMRN scientists are regularly invited to share their expertise and knowledge at conferences organized throughout Canada. During the past year, CBMRN scientists gave several presentations which greatly contributed to the reputation of the Network. They are listed in the Publications and Conferences section of this report.

Collaboration with the NMC

The worldwide web abounds with information on mastitis, but the majority is available only in English. The NMC, an international organization for the improvement of udder health and milk quality, is certainly one of the most credible and useful sources of information (www.nmconline.org). In order to make this information more accessible to the Canadian dairy industry, the NMC has allowed the CBMRN to translate their documents into French. The Transfer Manager is a member of the NMC Education Committee and one of the current priorities of this committee is to translate NMC documents so they are available to people around the world. The CBMRN Transfer Manager is the resource person and designated translator of NMC documents into French.

In 2007, the NMC created a section in their website where international surfers can find NMC translated articles in French and Spanish. The CBMRN Transfer Manager will continue to execute her role as a French translator of articles for 2008. The CBMRN will also help the NMC increase their international membership in French speaking countries.

User List

Users can register to be placed on the CBMRN electronic mailing list to receive the Mastitis-flash and the CBMRN Bulletin. Nearly 800 people are registered to date (about 500 Francophones and about 300 Anglophones). The list is composed of dairy producers, veterinarians, agronomists and advisers, students, and scientists in different regions of Canada. In 2007, the process of registering online was clearly improved by the creation of an interactive link found in the Mastitis: Resources Online section. With a click on that link, the user is guided to an online registration form where basic information is entered. This information is then electronically sent to the Transfer Manager to be processed.

Promotion of technologies

This section of the transfer program aims to promote the effective transfer of CBMRN technologies to the Canadian dairy industry. The process of protecting the intellectual property and marketing are the responsibility of research institutions. The CBMRN serves to support institutions and scientists in their quest to protect intellectual property and to maintain links with potential commercial partners. During fall 2007, potential commercial technologies have been identified by the CBMRN’s administrative team and communication was established with the associated research institutions and their researchers.

During the 2007 Annual Scientific Meeting, an Industry workshop was conducted for the benefit of Network scientist and pharmaceutical companies involved with vaccine development. Some of our researchers working in mastitis vaccine development presented their preliminary findings at the workshop. Novartis Animal Health presented their views about vaccine development now and in the future.
Impact Indicators

Evolution of the Knowledge Transfer Program

• $99,600 and $104,901 (Year Two and Year Three, respectively) are dedicated for knowledge transfer activities

• Development of a new tool – the Mastitis-flash – an electronic newsletter with monthly distribution to nearly 800 subscribers

• An electronic mailing list of about 800 people, an increase of more than 100 individuals from last year

• Canada, France, USA, Belgium, Argentina, The Netherlands, Morocco, and Algeria are the most active countries in accessing the CBMRN website

Availability of mastitis control and monitoring information for the Canadian dairy industry

• 3 transfer sheets were developed

• 16 conferences involved CBMRN participation

• 3 bulletins were distributed to about 900 people

• 5 Mastitis-flash newsletters were sent to about 800 people

• 23 articles about CBMRN activities were published

• 7 Mastitis Column articles were published

• 64 additional references were added to the website

• 60 CBMRN mastitis information packets were distributed to agricultural schools and 425 at colloquiums and conferences

Exposure to knowledge transfer strategies

• 7,265 visits on the CBMRN website over the period of April 2007 to March 2008 compared to 3,186 for the same period last year

• A total of 38,388 webpages consulted from April 2007 to March 2008 compared to 11,470 for the same period last year

• An electronic mailing list of about 800 people

Active uptake of mastitis control and monitoring information

• Approximately 2,500 people attended various conferences where CBMRN activities were presented

• About 50 requests to be placed on the electronic mailing list of the CBMRN were received

• An average of 489 people visited the CBMRN website every month
One of the main objectives of the CBMRN is to ensure that all trainees have an integrated multidisciplinary understanding of bovine mastitis. We currently supervise 21 graduate students and 8 postdoctoral fellows as well as 28 undergraduate students and 23 technicians. Most CBMRN students are co-supervised by at least one other CBMRN scientist in addition to their respective directors. Many of the co-supervisors are scientists from another research institution. This co-director approach to student training provides multidisciplinary understanding of mastitis and fosters resourcefulness and creativity in research.

Approximately 20% of the Year Two budget was directly devoted to the support of graduate students, postdoctoral fellows, undergraduate students, and training activities. Two graduate students are entirely supported by scholarships from external sources.

We train our students in a networking environment and provide them activities to promote networking. This was demonstrated during the CBMRN Annual Scientific Meeting in November 2007 where 78% of graduate students and 25% of postdoctoral fellows were in attendance. All graduate students present actively participated in the meeting where fifteen research posters and ten oral presentations were presented. A poster competition was held where four graduate students that were judged to have the best posters received a voucher for $500. The vouchers will be used to attend an international meeting to present their CBMRN research results. During the Annual Scientific Meeting, students interacted amongst themselves, with scientists, external experts, dairy producers, and industry partners. This gave them an opportunity to become familiar with the function of the Network. They also gained insight into the numerous research activities of Network scientists and were able to hear first-hand questions and concerns from dairy producers. Students indicated that their attendance at the meeting helped them keep up-to-date on research findings with respect to treatment, vaccination, and host response to various pathogens. The Annual Scientific Meeting also created an opportunity for students to meet their peers and to make contacts with researchers to discuss future studies and collaborations.

A training committee was formed this year to implement the training plan which includes inter-laboratory exchange programs, training modules, and student grants for participation in international scientific meetings. The inter-laboratory exchange allows CBMRN students to work in the laboratory of other CBMRN scientists for up to four months. This program permits them to learn new techniques, generate results for their projects and create new collaborations. The SNEI grant helped the training program by, among others, creating awards for international laboratory visits and international student internships. The latter allows for international students to work in CBMRN laboratories for up to 3 months.
An electronic mailing list is available to facilitate and promote collaboration and networking amongst CBMRN students. A section on our website is also dedicated to current and future students.

The CBMRN will continue to recruit highly qualified personnel to meet the needs of planned research and new initiatives. Additional graduate and undergraduate students will be starting their study within the Network during the coming months. The CBMRN experience will encourage trainees to take a holistic approach to science by understanding the interrelationship between fundamental and applied mastitis research.

“I was able to start a Ph.D. program after my master degree because CBMRN funding permitted me to extend my S. aureus virulence gene expression work in the mouse model to the cow level” Marianne Allard, CBMRN student (December 2007). Now that she has research experience in bovine mastitis, Marianne wants to continue her studies in this area, applying her molecular biology skills in bovine disease. This is a great example of how our multidisciplinary training program provides incentives and challenges to continue research on bovine health.

Left to right: Heba Atalla, University of Guelph; Signe Andersen, University of Prince Edward Island; Marianne Allard, Université de Sherbrooke.

Impact Indicators

Impact on the capacity for research and transfer - Highly qualified personnel trained in mastitis related activities

• 28 undergraduate students participate in CBMRN projects

• A total of 57 students and post-doctoral fellows received training during Year Two compared to 30 the previous year

• 1 student completed his Master of Science degree in the field of immune response to bacterial factors and 2 students completed their Ph.D. degree; one in the field of genomics and the other in the field of epidemiology

• One of the Ph.D. graduates is now working in an udder health related domain in The Netherlands, while the other is a post-doctoral fellow in horse genetics in the United States. The Master of Science graduate was accepted to the Ontario Veterinary College
The mission of the CBMRN is “to mobilize resources to reduce the impact of mastitis through concerted research and transfer of research results to users.” Networking allows us to pursue our mission by coordinating the use of existing resources to work toward common goals in mastitis research and to create resources that would not be possible without the CBMRN. The networking plan strives to maintain real links to our dairy industry partners and their priorities. The plan also strives to maintain a dynamic internal network of collaboration and communication, to maintain accessibility to Network infrastructure and technical expertise, to optimize transfer of results, technology, and knowledge among scientists, and to increase international visibility of Canadian mastitis research.

Networking within the CBMRN

A critical indicator of the performance and success of the Network is evidence of coordinated mastitis research throughout Canada. During the formation of the Network and in its operation today, numerous new collaborations were realized and existing partnerships among researchers have been strengthened. All researchers collaborate directly with at least two network scientists and most of CBMRN scientists have a working relationship with colleagues outside of their own province. Thirteen internal collaborations presently exist within the CBMRN in comparison to nine the previous year.

The major networking opportunity for our members every year is the Annual Scientific Meeting. This year, it was held on November 12 and 13, 2007 in Montréal, Québec. A record attendance of over ninety scientists, students, technicians, and partners was observed. Principal investigators of each research project presented a summary of their current results and activities. Additionally, our guest speaker, Dr. Stephen Oliver from the University of Tennessee, presented the latest information on an important environmental mastitis pathogen, *Streptococcus uberis*. The Annual Scientific Meeting was a highly successful networking activity. Next year, with the help of the SNEI grant, the CBMRN Annual meeting will be held jointly with the Mastitis Research Workers Conference (MRWC). It will be a four day event where day 1 and 2 will concentrate on CBMRN activities and day 3 and 4 will take the organizational format of the MRWC. The CBMRN scientists, students and technicians will be encouraged to participate in all sections of the meeting.
In order to discuss progress of projects or special challenges, several meetings by conference call or internet conferencing took place during the past year. Also, the list server groups were kept updated and frequently used to freely exchange ideas, comments, and suggestions on various topics. An inventory of CBMRN resources and facilities is accessible to all Network scientists and students in a secured section of our website. This is designed to facilitate the efficient use of expertise and infrastructure to expedite progress in research. Additionally, the Core Research Platform database is available to all CBMRN researchers.

**Networking with Partners**

The CBMRN is the realization of the dairy industry’s vision for a coordinated, efficient udder health research program that effectively transfers knowledge and technology to dairy producers. The involvement of our partners is fundamental to the Network’s vision and longevity. The main partner-networking activity of the past year was the 2007 Annual Scientific Meeting where twelve representatives of our industry partners were in attendance. They gained first-hand knowledge of the Year Two progress of each research project. During the presentation of research results and during a workshop session, our partners were able to voice questions and concerns directly to researchers. An excellent exchange of commentary and ideas took place.

Network news, activities, and events were distributed to partners in three issues of the CBMRN Bulletin. All partners can also access Network information via the CBMRN website. The CBMRN website is constantly updated and modified to make it more dynamic and to ensure the availability of great quantities of new, up-to-date information.

**Networking at the International Level**

In addition to internal networking and having an open dialog with industry partners, our networking plan is designed to promote linkages with international organizations. The plan encourages and fosters camaraderie, support, and collaboration with mastitis research groups outside of Canada. This plan introduces the CBMRN by promoting its research activities globally in order to establish new collaboration and to maintain existing links with international research groups. A significant part of the additional SNEI grant will be used to foster international linkage, provide awards to CBMRN trainees to attend international meetings, and award scholarships for international student exchanges. Travel grants will also be available to CBMRN scientists to meet with international mastitis networks or to invite international scientists to contribute to CBMRN activities.

During the past year, CBMRN members actively represented the Network at various conferences such as the Mastitis Research Workers Conference (November, Minnesota), the Heifer Mastitis Conference (June, Belgium), the 13th International Conference on Production Disease in Farm Animals (July, Germany), the International Symposium on Animal Genomics for Animal Health (October, France), the Conference of Research Workers in Animal Disease (December, Chicago), and the NMC 47th Annual Meeting (January, New Orleans). By having this exposure, members of the Network were able to form numerous contacts with international mastitis research organizations. This allowed for the establishment and coordination of current and future research activities with groups such as the Dutch Udder Health Board where meetings were held to exchange expertise in knowledge transfer and explore scientific collaboration. International exposure of the Network has also led to the recognition of the CBMRN as the Canadian point of contact for mastitis research and mastitis related issues through formal inclusion in the United States Department of Agriculture, Cooperative Multi-State Mastitis Research Project (N.E.-1281). Individual collaboration among research laboratories have been established or planned in Year Two of the CBMRN. These include participation in Network research activities by the University of Tennessee, University of Missouri, and the National Pintung University of Science and Technology, Taiwan. Twenty three international collaborations presently exist compared to three last year.
Impact Indicators

Impact on the capability and capacity of Canadian mastitis research

- Two Canadian scientists have joined the CBMRN and added mastitis research to their scientific focus
- Thirteen internal collaborations exist among CBMRN scientists compared to nine last year
- CBMRN scientists collaborated with twenty three external scientist compared to three last year
- $53,400 of new funding in Year Two has been obtained (NSERC Strategic Networks Enhancement Initiative) to build collaborations with international mastitis research groups. $51,000 of funding is conditionally available for Year Three
Introduction: The Research Program

Mastitis is a very complex disease that can have a significant economic impact on the dairy industry. The industry’s mission to maintain the quality and availability of dairy products depends on the implementation of an udder health management program that is based on sound mastitis research. A high level of coordination and collaboration is needed to administer a successful mastitis research program. The CBMRN responds to this need by the formation of an innovative partnership between scientists, the dairy industry, and NSERC. Our research program is designed to optimize scientific expertise in addressing dairy industry priorities with the goal of defining the mastitis situation in Canada, formulating novel mastitis solutions, delivering solutions to the industry in a timely manner, and establishing a foundation of networking to perpetuate research and transfer activities in the future.

The research program is composed of two themes areas, Mastitis Monitoring and Mastitis Control, which are linked by the Core Research Platform. This platform serves as a central asset of on-farm information and biological resources. The Themes’ research activities focus on highly specific mastitis issues that were put forth by industry partners and Network scientists.
Core Research Platform

Theme leader: Daniel Scholl, Université de Montréal

The Core Research Platform (CRP) is a window of opportunity for creating a platform that supports a diversity of mastitis research and links mastitis researchers with dairy farmers. This project offers a national-level program for coordinating data collection and data archiving. The goal of the CRP is to enhance on-farm mastitis data collection. This will enable and harmonize mastitis research by providing to a diverse group of scientists uniform data that originates from a single, nationally representative population of dairy farms. The specific objectives of the CRP are 1) to establish a National Cohort of Dairy Farms (NCDF) as the key source of data, 2) to form a Mastitis Laboratory Network (MLN) to analyse milk samples from the NCDF, and 3) to establish a Mastitis Pathogen Culture Collection (MPCC) that will store and preserve bacteria isolated from milk samples collected from the NCDF.

(1) The National Cohort of Dairy Farms (NCDF)

Coordinator: Daniel Scholl, Université de Montréal
Operations manager: Kristen Reyher, University of Prince Edward Island

The NCDF is a pan-Canadian cohort of commercial farms that is being followed over two years. Each farm is being monitored to determine and characterise the rate of intramammary infection and clinical mastitis. The NCDF also provides basic data needed for other CBMRN research activities. Regional cohort centres at the Universities of Calgary, Guelph (Kemptville Campus), Montréal (Saint-Hyacinthe Campus), and Prince Edward Island have been established. Four areas of activity in the NCDF are crucial to realising the CRP’s objectives: (a) establishing effective working relationships with recruited dairy farms, (b) development and utilisation of a CRP Reference Manual of standard operating procedures, (c) creation and maintenance of a CRP modular database, and (d) the creation of a DNA sample archive of cows in the NCDF.

(a) A total of ninety one dairy farmers and their respective veterinarians were successfully recruited to participate in the NCDF. Ninety percent of those farms were retained during this first year of data collection. Data collection started on farms in early 2007 and will continue until 2009. (b) A CRP Reference Manual was compiled and distributed to the regional coordinating centres. The Manual contains all of the NCDF standard operating procedures and protocols to ensure uniform data collection and processing among farms and coordination centres. This will ensure that the data collection needs of NCDF dependant research projects are fully met. (c) The CRP’s modular database serves as a tool for CBMRN scientists to access information on dairy herds enrolled in the NCDF. Information such as herd health management practices, udder health status of cows, and the identification of bacteria isolated from milk samples are readily available. The database software is adapted to meet specific CRP requirements and is modified to accept monthly electronic updates from the dairy herd improvement organisations (CanWestDHI and Valacta). The CRP database platform is installed at each regional coordinating centre. Over eighty-two thousand milk samples have been registered in the database during this first year of sample collection. (d) Protocols have been established for creating and implementing an archival system for a DNA bank of NCDF cows. This archive will serve as a permanent source of DNA from cows that have extensive management and health information.
(2) The Mastitis Laboratory Network (MLN)
Coordinator: Serge Messier, Université de Montréal

The MLN is an assembly of diagnostic laboratories that utilize standard operating procedures for identifying bacterial pathogens in milk samples. The procedures meet current laboratory standards for mastitis research and assure CBMRN scientists of uniform data from every laboratory. The MLN receive and culture all milk samples from the NCDF, identifies and records the 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. Year Two activities of the MLN consisted of implementing standard operating procedures for the identification of bacteria in milk samples that were received from the NCDF. Over eighty two thousand milk samples have been processed and recorded thus far.

The MLN is adding valuable capacity to Canadian mastitis research by establishing laboratories in key locations throughout the country. With the establishment of the MLN, current and future mastitis research will be easier to organize and implement due to the strategic, geographical distribution of diagnostic laboratories throughout Canada, and by the employment of standard operating procedures in each laboratory. In addition to the MLN laboratories at the University of Prince Edward Island, University of Saskatchewan, and the University of Montréal, work is progressing to establish another milk quality diagnostic laboratory at the University of Calgary. The CBMRN provides a critical mass of milk samples needed to establish these laboratories, which in turn become a durable resource for Canadian mastitis research.

(3) The Mastitis Pathogen Culture Collection (MPCC)
Coordinator: Grant Tomita, Université de Montréal

While mastitis epidemiology researchers are more inclined to use numerical data from the NCDF, other scientists will use bacterial isolates in their research. The MPCC is the CRP vehicle that provides access to bacterial pathogens isolated from udder and bulk tank milk samples. In this second year of the Network, the objectives of the MPCC were (a) to implement standard procedures for the accession and distribution of bacterial isolates and (b) to continue to build inventory of the culture collection. Procedures for submitting, conserving, and distributing isolates are established and in place. Isolates are readily available to CBMRN researchers and play a critical role in Network projects which investigate pathogen virulence factors and antibiotic resistance. We actively promote mastitis research outside of the Network by encouraging non-Network scientists, both domestic and international, to access the MPCC. A particular strength of the MPCC is that isolates are fully cross-referenced with all NCDF data. This includes epidemiologic and demographic data associated with cows and farms, and physiology data and archived DNA samples of cows. Bacterial isolate information will be available through the CBMRN website, CBMRN Bulletin, or by personal contact with CBMRN scientists. Over fourteen thousand isolates have been conserved in the culture collection to date.

Although the start-up of the CRP was initially behind schedule as a consequence of conflicts in operational scheduling and the sheer immensity of the undertaking, the Platform is now fully operational and prepared to undertake the second year of data collection in the third year of the Network research plan. The entire infrastructure and procedures to implement the CRP are in place. The CRP builds capacity of research and technical service to the industry by training highly qualified students and technical personnel. The cadre of laboratory and field technicians trained in mastitis-related work through their employment in the CRP serves as an important resource for mastitis research and related dairy health projects in Canada. Frequent contact among regional coordinators, dairy farmers, and veterinarians allows for the timely transfer of cow health information as well as current and new mastitis monitoring and control knowledge.
Mastitis Monitoring Theme

Theme leader: Herman Barkema, University of Calgary

The most important goal of a mastitis management program is to substantially reduce the occurrence of clinical mastitis and subclinical intramammary infection. Sound udder health decisions that are based on mastitis monitoring at the level of the cow, herd, and industry can contribute significantly to reaching this goal. The Mastitis Monitoring Theme’s goal is 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. Specific objectives of the Monitoring Theme are 1) to develop novel strategies to optimize the monitoring of udder health, 2) to characterize virulence factors of major mastitis pathogens, and 3) to develop rapid methods for detecting mastitis pathogens.

The Research Projects

Investigating the Occurrence and Monitoring of New Udder Infections

Principal investigator: Ian Dohoo, University of Prince Edward Island

Mastitis is caused by a variety of bacteria, and risk factors for infection differ among these bacteria. Therefore, to truly describe the dynamics of new udder infections, bacteriological culture of milk samples to identify the cause of infection are required. Many sources of information to indicate udder infection in a dairy herd are readily available. These include individual cow and bulk tank somatic cell counts, and bacteriological culture of composite samples which contain milk from all four quarters. Although the information gathered by somatic cell counts and composite milk samples can be useful, they are not as precise as bacteriological culture results from frequently sampled, individual quarters. The bacteriological culture of quarter milk samples taken frequently over time will indicate dynamics of new udder infection, which is very important at the level of the dairy herd. However, milk culturing can be expensive and time consuming. Therefore, a need exists for more efficient methods to monitor the dynamics of new udder infection at the herd level. A combination of sources of udder health information may serve as a suitable alternative for the bacteriological analysis of frequent quarter milk samples. Udder health data is available, but at times it is not utilized to its full potential. Therefore, the goal of this project is to optimize the use of these data for monitoring udder infection at the herd level. The large scale NCDF project provides an opportunity to measure mastitis incidence levels in Canadian herds. This project will (a) determine the frequency of new udder infections and identify bacterial species that cause infections, (b) test alternate methods for monitoring the frequency of new udder infections, and (c) differentiate risk factors that influence the frequency of new udder infection and risk factors that contribute to chronic infection.

(a) 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 NCDF and the testing of samples continues. (b) Results from a series of studies to determine the validity of using frozen milk samples for determination of somatic cell counts (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. (c) Three methods to capture on-farm mastitis monitoring and control practices were developed and are being analysed to determine effects that contribute to the frequency of new and chronic mastitis.
Identifying Genes That Enable Bacteria to Cause Chronic Intramammary Infections and Clinical Mastitis

Principal Investigator: François Malouin, Université de Sherbrooke

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 mastitis. It is important to identify these genes as they could be useful in developing diagnostic tools for identifying problematic *S. aureus* mastitis and for identifying antigens for vaccine development. The specific objectives of this project are (a) to compare and identify the virulence genes found in bacterial isolates that cause chronic mastitis and (b) to identify genes that are specifically expressed by *S. aureus* in the mammary gland during mastitis.

This project utilizes DNA array technology to reveal genetic relatedness among various strains of *S. aureus* that cause mastitis. (a) To date, forty eight *S. aureus* mastitis isolates were genetically characterized. Eleven out of the forty eight isolates were collected from the same cows 60 days apart (between dry-off and calving) and were considered chronic infections. Results thus far have shown that all isolates belong to seven genetically distinct clonal types and ten of the eleven isolates that caused chronic mastitis were confirmed to share identical genetic markers. (b) *S. aureus* DNA microarrays were developed and utilized to identify certain genes that are expressed during mastitis. These molecular markers have the potential to be used as diagnostic tools for the rapid identification of *S. aureus* mastitis and may also represent excellent candidates for vaccine antigens. Results to date suggest that a specific set of virulence genes predisposes some isolates of *S. aureus* to cause chronic mastitis and the regulation of their gene expression during mastitis influence the type of infection – subclinical or clinical mastitis.

Accomplishments in Monitoring Theme Research, Networking, and Training

Excellent progress has been made in Year Two of the Mastitis Monitoring Theme. Both projects have added new trainees and are executing the research as planned. The project that is investigating the genetic characterization of virulence factors is providing training in mastitis molecular biology research for one M.Sc. student, one Ph.D. student, three undergraduate students, and one postdoctoral fellow. An additional M.Sc. student is receiving training in mastitis pathogen molecular epidemiology through this research. New collaborations among molecular biologists, a clinician, an agronomist, and an epidemiologist have developed through this research. Preliminary results from this Theme were transferred to CBMRN scientists and dairy industry partners in six posters at the 2007 CBMRN Annual Scientific Meeting. Two posters were also presented at the Annual Meeting of the American Society for Microbiology and five posters were presented at dairy producer meetings. One manuscript was published in a dairy producer magazine and two manuscripts are being prepared for submission to peer-reviewed scientific journals.
Theme Leader: Pierre Lacasse, Agriculture and Agri-Food Canada

Although much progress has been made in dairy health management practices, mastitis continues to be a major concern of the dairy industry. Numerous management practices are promoted as ways to prevent mastitis, but those practices do not always reduce the occurrence of intramammary infection. The research program of the Mastitis Control Theme focuses on augmenting and improving those practices with the development of knowledge, new practices, products, and technology to reduce intramammary infection and clinical mastitis. Specific objectives of this research theme are 1) to enhance the cow's resistance to mastitis pathogens, 2) to develop therapeutic strategies to control this disease, and 3) to evaluate the association between mastitis treatment and bacterial resistance to therapy.

The Research Projects

The use of Genetic Profiling to Identify Cows with Enhanced Immune Defence against Mastitis Pathogens

Principal Investigator: Bonnie Mallard, University of Guelph

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 defence following exposure to mastitis pathogens. This knowledge can be used as a means to improve animal health and food-safety. This project explores the genetic regulation of the bovine immune response to mastitis pathogens by (a) identifying cows with a high or low immune response, (b) determining how the genetic profile of high and low immune responders are associated with resistance or susceptibility to mastitis, and (c) identifying novel proteins in resistant and susceptible cows and identifying specific genetic sequences that are associated with the proteins.

(a) Protocols have been developed and are being implemented for the identification of high and low immune responder cows that are enrolled in the NCDF. Immune response in association with health and production traits is being analysed. About 500 cows will be tested and hair samples from those cows are being collected as a source of DNA. (b) 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. (c) Methods are being developed to identify and analyse 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.
Proteins in Milk and Resistance to Clinical Coliform Mastitis

Principal Investigator: Xin Zhao, McGill University

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 of this project are (a) to characterize the association between CD14 and resistance to clinical coliform mastitis and (b) to produce CD14 in transgenic sheep and determine the role of CD14 during coliform mastitis.

(a) 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. (b) 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.

Mastitis Vaccines

Mastitis vaccines have the potential to enhance the cow’s resistance to intramammary infection and can be a useful tool in supplementing established udder health management programs in the prevention and control of mastitis. Currently, there are no vaccines on the market that guarantee the total control of common and virulent strains of mastitis-causing pathogens. The aim of the following three projects is to develop vaccines that enhance resistance to mastitis, and therefore have the potential to reduce the use of antibiotic therapy.

Development of a DNA Vaccine against *Staphylococcus aureus* Mastitis

Principal Investigator: Brian Talbot, Université de Sherbrooke

The complete genomic sequences of *S. 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 of this project are (a) to identify a combination of antigens and adjuvants that could be used as a vaccine to protect against *S. aureus* mastitis, (b) to evaluate the efficacy of several vaccine formulations in mice, and (c) to evaluate the immune response and efficacy of optimized vaccine formulations in cows.
(a) Four *S. 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. (b) In order to test the efficacy of the protein antigen-plasmid adjuvant vaccine, mice were immunised with the formulation and then experimentally infected with *S. aureus*. The results clearly showed that mice were protected by the protein-plasmid formulation. (c) This vaccine formulation is currently being tested in a challenge trial with cows. The trial is on-going and results are not yet available.

**Development of a Vaccine against Coliform Mastitis**

Principal Investigator: Grant Tomita, *Université de Montréal*

The success of a vaccine against clinical coliform mastitis requires elevated and sustained antibody production during the lactation cycle when cows are most susceptible to infection. Commercially available coliform mastitis vaccines have been developed to increase the cow’s antibody levels and these vaccines have been shown to reduce the duration and severity of clinical coliform mastitis. However, the vaccines must be repeatedly injected in order to achieve a high level of antibody production. Administration of the vaccines is labor intensive and has the potential to compromise the health and welfare of cows. The microencapsulation of vaccine antigens in biodegradable polymers can serve as an alternative to conventional vaccine administration protocols. The polymer serves as a vehicle for the controlled release of vaccine antigens after administering a single injection which results in prolonged antibody production. To avoid the issue of multiple shots and intensive animal handling, a single shot coliform mastitis vaccine is being developed in this project. The objectives are (a) to optimize microencapsulation techniques in the synthesis of an *E. coli* J5 bacterin, (b) to evaluate the immune response to the optimized vaccine, and (c) to initiate a challenge trial to determine the efficacy of the optimized vaccine.

(a) 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. (b) Cows were vaccinated once with the optimal formulation and were observed to have a similar antibody response as that of cows which received a commercially available vaccine that required multiple shots. (c) A challenge trial to determine efficacy of the single shot, microencapsulated J5 vaccine is being planned and will commence in Year Three.
Vaccination Strategies to Enhance Immunity against *Staphylococcus aureus*

Principal Investigator: Andrew Potter, VIDO, University of Saskatchewan

Most *S. 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 *S. aureus* mastitis. Specific objectives include (a) optimizing the magnitude of immune response, (b) optimizing local mammary gland immunity, and (c) testing a nucleic acid vaccine.

(a) Year One results of this project demonstrated that a vaccine formulated with proteins from the cell wall of *S. aureus* (GapC/B proteins) induced a significant increase in antibody production in mice. In Year Two of this study, cows were vaccinated with the GapC/B proteins and exhibited an excellent immune response to the antigens. (b) 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. (c) 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.

Antibiotics and Mastitis

Since the introduction of antimicrobial drugs in the 1940’s, their impact in human and veterinary medicine has been dramatically altered by the development of resistant bacteria. In addition to concerns regarding the impact on animal health, there is growing concern of the spread of resistant bacteria to the human populations through contaminated food and the environment. 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 following projects address the issues of when is it appropriate to use antibiotic therapy and if antibiotic therapy has an effect on bacterial resistance in the treatment of mastitis.

Validation of On-farm Mastitis Pathogen Identification Systems

Principal Investigator: Greg Keefe, University of Prince Edward Island

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. The CBMRN provides the opportunity to conduct a large-scale study to compare on-farm diagnostic tools and their ability to influence decision protocols for antibiotic therapy. This project will test 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 (a) examined five 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 (b) 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 NCDF. Data generated from the second phase will allow for the formulation of rules for the treatment of mastitis.

(a) Two identification tools, Petrifilm™ and Biplate™ media systems were found to exhibit excellent performance characteristics in the rapid identification of mastitis pathogens in milk samples. Each system was deemed to be adequate for on-farm use. (b) 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.

Investigating the Association between Antimicrobial Treatment and Antimicrobial Resistance in Mastitis Pathogens

Principal Investigator: Herman Barkema, University of Calgary

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. Therefore, the objectives of this project are (a) to determine antimicrobial use in Canadian dairy herds, (b) to determine antimicrobial resistance of mastitis pathogens isolated from Canadian dairy herds, and (c) to describe changes in occurrence of resistant pathogens and its association with the antimicrobial use on dairy herds. Dairy farms that are enrolled in the NCDF will be monitored for two years to fulfill the objectives.

(a) 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

1 Minnesota Easy Culture System, University of Minnesota, Saint Paul, Minnesota.
participating farms. Receptacles are being collected monthly and the contents recorded. Information from this first year of data collection is currently being compiled. (b) Approximately seven hundred bacterial isolates from the MPCC have been selected and are undergoing antimicrobial resistance testing. At least two thousand \( S. \ aureus \) isolates and one thousand \( E. \ coli \) isolates will be evaluated for antimicrobial resistance by the end of this study. (c) 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.

**Accomplishments in Control Theme Research, Networking, and Training**

During the second year of the Network, seven postdoctoral fellows, eleven highly qualified graduate students, and two undergraduate students have worked on Control Theme projects. They serve as the driving force in all of this Theme’s research activities. Research projects are well on their way and results are emerging. These results have been published in seven research articles and five additional manuscripts are under peer-review. Eight oral presentations and five posters have been given by Control Theme researchers and students at domestic and international scientific meetings. In addition, nine posters were present at the 2007 CBMRN Annual Scientific Meeting. New internal and external collaborations are in place and two projects have leveraged additional external funding.
Impact Indicators

Impact on the capacity of Canadian milk bacteriology laboratories

- One new milk bacteriology laboratory established and plans are underway to establish still another laboratory
- Technicians from the three bacteriology laboratories are trained in high-throughput, NMC-based milk bacterial culture and species identification protocols
- Eighteen technicians trained to perform milk bacterial culture

Impact through actionable research that is accessible and actionable at the farm-level

- A CBMRN knowledge transfer program is gearing up for the dissemination of early results from three CBMRN research projects and research collaborations
- Three new applied research projects were initiated as a direct result of the CBMRN program
- 33% of the CBMRN research budget was attributed to applied research (2006-2008)
- Canadian dairy industry investment in applied research projects was leveraged by a factor of nine ($total / $industry) (2006-2008)
- Knowledge from CBMRN applied research was internationally disseminated in 1 scientific article and 16 research presentations (2006-2008)

Impact through developmental research - Mastitis monitoring and control technologies

- 67% of the CBMRN research budget was attributed to technology research (2006-2008)
- Canadian dairy industry investment in technology research of the CBMRN was leveraged by a factor of 7.8 ($total / $industry) (2006-2008)
- Knowledge from CBMRN technology research was internationally disseminated in fourteen scientific articles and twenty two research presentations (2006-2008)

Impact of the National Cohort of Dairy Farms

- Impact will be measured during 2008-2009 and 2009-2010
Cash Contributions

- NSERC $2,577,342 (78%)
- Partners $421,668 (13%)
- Balance Year 1 $288,298 (9%)

Total of contributions $3,287,308 (100%)
Total of expenditures $2,751,892 (100%)
Balance $535,416

Expenditures (Amounts based on estimations)

- Research $1,898,237 (69%)
- Training $574,719 (21%)
- Networking-Transfer $74,511 (3%)
- Administrative Centre $204,425 (7%)

Total of expenditures $2,751,892 (100%)
DEVIATIONS FROM OBJECTIVES AND PLANS FOR NEXT YEAR

The CBMRN research program is on-track as planned. While some individual projects have experienced minor delays, there are no deviations from our global or specific objectives. Although the initiation of several formal components of our Training Program is behind schedule, we expect to catch up during the coming year. The positive 2007-2008 year-end balance (16% of the 2007-2008 budget) is the net result of these delays. We carry this balance forward with confidence that the funds will be well used to reach all original objectives. In Year Three, we will continue to fully realize the implementation of the Core Research Platform as a hub of information and resources. The National Cohort of Dairy Farms, Mastitis Laboratory Network, and Mastitis Pathogen Culture Collection components of the Core Research Platform will once again set the tone for advancements in Theme area research and in the education and training of highly qualified personnel. Numerous projects within the Monitoring and Control Themes will continue to mature and they are anticipated to resume output of highly relevant knowledge and technology for dissemination to our industry partners.
MONITORING MASTITIS RESEARCH AROUND THE WORLD

The main purpose of “Monitoring Mastitis Research Around the World” is to get a better understanding of global mastitis research activities. Information from this section will be presented under various formats, including a computerized directory, a report, and in a section entitled “Mastitis around the World” on our website.

International directory of mastitis researchers

This year, emphasis has been placed on validating and organizing information gathered in the previous years while building the “International Directory of Mastitis Researchers”. This directory is formatted as a database found under the “Research” section on our website.

To restrict the size of the directory, sixteen countries representing four continents were recognized as being the most active in mastitis research. For each selected country, only researchers who are the principal author or co-author of scientific articles published between 2003 and 2008 were selected. We have prioritized about twenty-five researchers per country who had 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, England and Germany, the directory may contain information on fifty to a hundred researchers.

Information about researchers will be classified into two categories according to research interest: mastitis control – which includes management, milking procedures and equipment, therapy, and immunity subcategories, and mastitis detection and diagnosis – which lists somatic cell count, milk components, bacteriology, and pathogens subcategories. Scientists can also be cross referenced by the particular pathogen that is associated with their research.

This tool will promote CBMRN collaboration and communication with the international scientific community. The directory will be available to CBMRN members and partners through the electronic mailing list and will be featured on the CBMRN website in the spring of 2008.

Mastitis Around the World

“Mastitis Around the World”, found under the “Mastitis: Online Resources” section on our website is now available to all Internet surfers. 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 will give a description of “Who are they?” and “What do they do?” Links with their latest publications and website, if available, will also be included on the “In brief” page. By the summer of 2008, a report will be prepared to identify areas of mastitis research that are being prioritized by international mastitis researchers. The focus will be directed toward countries included in the directory, for each country, major research groups will be listed and their principal categories of research interest or research expertise will be identified.
### Mastitis Column

**In « Le Producteur de lait québécois »**

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<td>February 2008</td>
<td>Le traitement au tarissement, Y a-t-il un risque réel d’antibiorésistance?</td>
<td>E. Poirier, D. Scholl and A.-M. Christen</td>
<td>Dry-cow treatment and antibiotic resistance</td>
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<td>July/August 2007</td>
<td>Mammite et antibiorésistance</td>
<td>D. Scholl, H. Barkema and J. Baillargeon</td>
<td>Testimonial of a dairy producer about antibiotic resistance – Presentation of related CBMRN research projects</td>
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<td>April 2007</td>
<td>Sur la piste d’un vaccin contre la mammite à <em>Staphylococcus aureus</em></td>
<td>C. Ster, B. Talbot, P. Lacasse and P. Bilodeau</td>
<td>Developing tools, as vaccine, to better fight against contagious pathogens</td>
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### In « The Milk Producer »

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<tr>
<td>March 2008</td>
<td>Still a safe bet for healthy udders</td>
<td>E. Poirier, D. Scholl and A.-M. Christen</td>
<td>Dry-cow treatment and antibiotic resistance</td>
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<td>November 2007</td>
<td>Rational antibiotic use</td>
<td>D. Scholl, H. Barkema and J. Baillargeon</td>
<td>Testimonial of a dairy producer about antibiotic resistance – Presentation of related CBMRN research projects</td>
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<td>April 2007</td>
<td>On-farm benefits</td>
<td>J. Baillargeon</td>
<td>Testimonials of Ontario dairy producers on their participation in several research projects</td>
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Scientific Publications


Other publications


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<tr>
<td>Heifer Mastitis Conference</td>
<td>2007 June 24-26, Ghent, Belgium</td>
<td>H. Barkema</td>
<td>Impact of heifer mastitis: association with somatic cell count, production, culling and fertility</td>
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<td>Heifer Mastitis Conference</td>
<td>2007 June 24-26, Ghent, Belgium</td>
<td>J.-P. Roy</td>
<td>Evaluation of the California Mastitis test as a pre-calving treatment selection tool for Holstein heifers</td>
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<td>13th International Conference on Production Diseases in Farm Animals</td>
<td>2007 July 29-August 4, Leipzig, Germany</td>
<td>N. Karrow</td>
<td>Integrative immunogenomics and health of the dairy cow: SNPs, chips and latte to go</td>
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<td>NMC Regional Meeting</td>
<td>2007 August 9-10, Charlottetown, PEI</td>
<td>H. Barkema</td>
<td>The Canadian Udder Health Situation: Results of a Large National Study</td>
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<td>Maritime Quality Milk Advisory Board Meeting</td>
<td>2007 August 31, Charlottetown, PEI</td>
<td>J. McCarron</td>
<td>On-Farm Diagnosis of Mastitis The road to intelligent treatment decisions</td>
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<td>International Symposium on Animal Genomics for Animal Health</td>
<td>2007 October 23-25, Paris, France</td>
<td>B.A. Mallard</td>
<td>What are the critical needs and future applications in animal health?</td>
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<td>Mastitis Research Workers Conference</td>
<td>2007 November 7-8, Minneapolis, USA</td>
<td>D. Scholl</td>
<td>Canadian Bovine Mastitis research Network Progress Report: Core Research Platform</td>
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<td>2007 November 7-8, Minneapolis, USA</td>
<td>S. Dufoeur</td>
<td>Risk Factors Associated with Subclinical IMI Incidence on Canadian Dairy Farms</td>
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<td>Conference of Research Workers in Animals Disease</td>
<td>2007 December 2-4, Chicago, USA</td>
<td>C. Ster</td>
<td>Preliminary evaluation of IsdH as an antigen for vaccination against bovine S. aureus mastitis</td>
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<td>Colloque sur la santé des troupeaux laitiers</td>
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<td>H. Barkema and R. Olde Riekerink</td>
<td>La réalité de la mammite au Canada, où vous situez-vous?</td>
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<td>J.-P. Roy</td>
<td>Atelier mammite : Diagnostic rapide et traitement de la mammite clinique</td>
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<td>NMC 47th Annual Meeting</td>
<td>2008 January 20-23, New Orleans, USA</td>
<td>H. Barkema</td>
<td>Biosecurity and Mastitis</td>
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<td>Dairy Research Communication &amp; Extension Event</td>
<td>2008 February 19-20, Guelph, ON</td>
<td>T. DeVries</td>
<td>Update on the Canadian Bovine Mastitis research Network Ontario Cohort Study</td>
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<td>Western Canadian Dairy Seminar</td>
<td>2008 March 4-7, Red Deer AB</td>
<td>H. Barkema</td>
<td>New approaches to mastitis prevention</td>
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Scientific Posters


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