

# Ontario Food Safety Research Program Compendium



2004-2005

# PREFACE

Today's investments in food safety research will result in tomorrow's savings in health-care and industry costs and in an increased ability for Ontario to compete on the world stage. The Ontario Ministry of Agriculture and Food (OMAF) is dedicated to making Ontario's Food Safety System world-class and Ontario's food supply among the safest in the world. For these reasons OMAF is investing in state-of-the-art food safety research.

We are pleased to announce the funding of ten new research projects under the *Food Safety Research Program* (FSRP) that will advance our knowledge about the prevalence, prevention and detection methods of food-borne hazards. With these new projects we are investing close to \$0.7 million dollars in research projects being performed at four research institutions across Ontario: the University of Guelph, the University of Toronto, the National Research Council and the Public Health Agency of Canada. This provincial investment has leveraged an additional \$2.3 million in financial and in-kind support from research partners. Furthermore, the program has contributed to the training of highly qualified personnel by funding a total of 25 Ph.D., M.Sc. and B.Sc. students.

This was the fifth year of the competitive *Food Safety Research Program*, and it builds on the achievements from previous years, in which the FSRP has invested \$4 million for 34 research projects.

The FSRP has been successful not only in attracting excellent research and in achieving its stated objectives, but also in fostering collaboration in food safety research, and in disseminating results of research promptly and widely.

For additional information on OMAF food safety research we encourage you to visit our website at: [www.omaf.gov.on.ca](http://www.omaf.gov.on.ca).

For further information on any specific projects listed in this *compendium* you are encouraged to contact the lead researcher directly.

Finally we would like to recognize and thank the many researchers, universities, federal and provincial government departments and industry organizations that partner with OMAF to fund, perform and to communicate research results. We are excited about the new opportunities that have been identified, and we thank the many research institutions that have come together to define and carry on excellent, multidisciplinary food safety research. There are few things as important as the safety of our food and the research projects supported by FSRP continues to help enhance its safety for the benefit of Ontario citizens.

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# TABLE OF CONTENTS

<b>Preface</b> .....	<b>2</b>
<b>Section One:</b> <b>Food Safety Research Program 2004/05</b> .....	<b>4</b>
<b>Section Two: Abstracts</b> .....	<b>7</b>
Detection Methodology .....	7
Risk Assessment .....	11
Risk Management and Control .....	13
<b>Section Three:</b> <b>Status of Previously Funded Projects</b> <b>(2000/01 - 2003/04)</b> .....	<b>17</b>
<b>Section Four: Quick Searches</b> .....	<b>19</b>

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# Section One: Food Safety Research Program 2004/05

In this *Compendium*, we present the outcome of the *Food Safety Research Program* (FSRP) 2004/05 grant application and funding process.

For your convenience this *Compendium* is divided into four sections to allow greater search capabilities and ease of information dissemination.

## **Section One: Food Safety Research Program 2004/05**

In this section you will find the program description, update, research priorities, details about the latest program competition and interesting statistical information.

## **Section Two: Abstracts**

This section contains detailed abstracts of the projects categorized into three areas: Detection/validation methodology (DM); Risk Assessment (RA) – identification of emerging food safety hazards and contaminants; Risk Management (RM) of food safety risks. These abstracts include contact information for the lead researcher, name of collaborating researchers and project duration. In addition, for each project you will find a brief description of anticipated benefits to the food safety system in Ontario.

## **Section Three: Status of Previously Funded Projects (2000/01 – 2003/04)**

This section provides a snapshot of all funded projects since the beginning of the program with reference to the status of the project.

## **Section Four: Quick Search**

This section allows you to cross reference key words (e.g. *Salmonella*) to supported projects.

## **Background**

Ontario is recognized throughout the world for the quality and safety of its agri-food products. To retain this position of leadership in food safety, the province has initiated science-based, field-to-fork food safety system improvements. In partnership with the Ministry of Health and Long-term Care (MOHLTC) and the Ministry of Natural Resources (MNR), the Ontario Ministry of Agriculture and Food (OMAF) led an Ontario food safety system review. During the review process, OMAF recognized the need to update its standards and requirements to keep pace with changes in scientific information, technology, consumer behavior, consumer lifestyles and industry practices. The review was designed to improve Ontario's food safety system by increasing the government's capacity to maintain high standards of food safety, protect public health and increase the marketability of Ontario food products. The overall goal is to build a:

- modernized, science-based food safety system founded on the principles of risk analysis/risk management;
- seamless system that covers the food chain from field to fork;
- market-friendly system consistent with Ontario's trade responsibilities and industry needs.

## Program description

The *Ontario Food Safety Research Program* is a competitive research fund established in 2001 and funded on an annual basis. The mandate of the program is to fund innovative food safety research projects that enhance the safety of Ontario's food through:

- Development and/or validation of testing methods for the detection of pathogens and chemicals for use in laboratory and field settings;
- Identification of emerging food hazards and contaminants;
- Risk analysis, risk assessment, risk management and control in food safety.

Ultimate results of the program are new and/or enhanced technologies and diagnostic tools that support the agri-food industry and government regulatory and laboratory programs; new knowledge about emerging food hazards and contaminants; new strategies to reduce, eliminate and manage food safety risks. These results contribute to and support the implementation of HACCP and quality assurance programs throughout the food chain.

A Research Requirements Document is issued on an annual basis to solicit research proposals from academia, industry, and government or partnership networks with demonstrated capability to perform quality research in their area of expertise. For the 2004/05 competition the researchers were eligible to apply for up to \$100,000 per project that has to be completed within two years. The program strongly encourages the applicants to demonstrate extensive collaboration and secure matching funding if possible. OMAF staff and external peer reviewers review submitted proposals. Under the 2004/05 competition we received 40 Letters of Intent in response to our June, 2004 Call for Proposals.

The full abstracts of these newly funded projects, as well as those from previous FSRP funding cycles, can be found on our Web site:  
[www.omaf.gov.on.ca](http://www.omaf.gov.on.ca).

With these new projects we are investing almost \$0.7 million dollars for research being performed at four research institutions across Ontario, including the University of Guelph, the University of Toronto, the National Research Council, and the Public Health Agency of Canada.

It is important to highlight that from a total investment of \$0.7 million, 90% goes directly to support research and only 10% are the administrative costs.

To be successful in obtaining the program funds the researchers must satisfy the following program criteria:

- Fit to the research priorities described in a research requirements document; the relevance to current issues in food safety; the anticipated contribution to improving the food safety system in Ontario;
- Quality and clarity of experimental design and project work plan;
- Research capabilities of the researcher and establishment;
- Contribution from collaborators and the impact on the quality of research attained;
- Appropriateness of budget items - project costs must be reasonable and detailed;
- Effectiveness of the technology transfer and communications plan in facilitating the adoption or commercialization of the research results.

Overall the project proposals should:

- Foster innovative ideas (i.e., new detection methods, new strategies to reduce, eliminate, manage food safety risks)
- Nurture collaboration and synergy between food safety scientists, government agencies, policy makers and the industry
- Complement, build on, and/or feed into, but not duplicate the research programs of other funding agencies interested in food safety
- Encourage multi-disciplinary, collaborative participatory research
- Allow researchers to explore speculative 'high reward' opportunities
- Bring new researchers into food safety research
- Encourage special topics not well covered by other funding agencies

### Statistical Summary:

#### Overall FSRP Funding in 2004/05

- Letters of Intent received = 40
- Letters of Intent invited to submit full proposal = 20
- Applications offered funding = 10
- Success rate = 5%

#### Applications and Awards by FSRP Priority Area:

Priority Area	# Applications	#Grants Awarded
Development and Validation of Testing Methods (DM)	15	4
Risk Assessment (RA)	17	2
Risk Management (RM)	8	4

#### FSRP 2004/05 Funding Highlights

- Four awards focus on innovative, exploratory, and high-risk/high reward research projects in development of a novel approach in detection methodology by focusing on development and/or validation of biosensors, microarrays and PCR technology.
- Two awards investigate risk assessment – one is focused on determining a prevalence of food-borne pathogens on retail raw meat in an Ontario county; the other is focused on finding a model to utilize existing knowledge and to determine the best possible alternatives to manage food safety hazards.
- Four awards deal with innovative approaches to eliminate, reduce and manage food safety hazards, such as use of bacteriophages to destroy *E. coli* O157:H7 in cattle, use of feed supplements, xylanase to reduce *Campylobacter jejuni* colonization in poultry, use of a combination of UV and Hydrogen Peroxide to decontaminate minimally processed fruits and vegetables, and use of water acidifiers to reduce the prevalence of *Salmonella* in pigs.

The FSRP wishes to acknowledge and thank the peer reviewers who participated in the program review process for their service and dedication to the Program.

# Section Two: Abstracts

## Detection Methodology

# DM

### DM1: Improvement and Validation of a Novel Protein Microarray Assay for *Salmonella* Serotyping

#### Abstract:

A rapid, cost-effective and easy-to-use protein (antibody) microarray assay has been successfully developed for *Salmonella* serotyping by the Kauffmann-White scheme in our previous project. The assay involves immobilization of *Salmonella* antibodies onto epoxy activated glass slides, labelling of *Salmonella* cells with the fluorescent dye Eosin Y, capturing *Salmonella* cells by the antibodies, and detection of the fluorescent signal using a microarray scanner. A prototype antibody array was constructed for identification of 20 commonly identified and clinically important *Salmonella* serovars. The antibody array was able to detect multiple *Salmonella* O antigens, and both phase 1 and 2 flagella antigens simultaneously, and thus allowed correct one-step identification of the serovar. The assay was evaluated for *Salmonella* serotyping using 117 target and 73 non-target *Salmonella* strains belonging to 58 serovars. The microarray profiles allowed correct serovar identification of 86 target strains, and correct identification of O and most of the phase antigens for an additional 30 target strains. The assay also allowed exclusion of the 73 non-target strains from the 20 target serovars. Because of its speed, accuracy, low cost, and ability to identify both flagellar antigens simultaneously, the antibody microarray-based assay is a promising alternative to the current slide agglutination method for *Salmonella* serotyping. However, the assay needs further optimization, validation under applied conditions and standardized data analysis method/tool for its implementation in diagnostic laboratories.

In this project, we will improve and validate the system to make it operational by diagnostic labs. More specific antibodies and additional antibodies will be evaluated and included on the microarray to minimize impact of cross-reactions, and to allow exclusion of closely-related serovars. A customized software program will be created to facilitate data

analysis, interpretation and reporting. Sufficient antibody arrays will be produced and the system will be validated using at least 1000 *Salmonella* strains. The validation will be conducted side-by-side with the standard slide agglutination method under applied conditions in collaboration with the OIE Reference Laboratory for Salmonellosis, Public Health Agency of Canada (PHAC).

#### Expected Impact of Project Outcomes on Food Safety in Ontario:

*Salmonella* consists of over 2500 serovars. Serotyping is the most important universal typing method for characterization of *Salmonella* isolates, and involves more than 250 antisera for identification of all serovars. The current *Salmonella* serotyping method only allows for detection of a single antibody-antigen reaction at a time. A minimum of three to six antibody-antigen reactions are needed for recognition of a particular *Salmonella* serovar. The number of reactions required can be many times greater if a less common serovar is tested. The assay consumes high volumes of reagents, and takes three days to two weeks to provide an answer.

Successful completion of our proposed project will result in an improved and validated protein microarray-based test that can be used for rapid and cost-effective serotyping of *Salmonella* in a single test. This will allow testing laboratories and government inspection staff to conduct risk assessment and outbreak studies in a cost-effective and timely manner in support of monitoring and surveillance activities and enforcement of regulatory programs. The method developed in this project can be extended to serotyping of other food-borne pathogens such as *Campylobacter*.

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## DM2: Rapid Ultra-Concentration Technologies for Isolation of Pathogen DNA and RNA Markers from Foodstuffs for On-line Detection and Screening

**Abstract:**

Two key challenges that must be overcome prior to the implementation of practical biosensor and biochip technologies for testing of food and water samples for nucleic acids indicative of pathogenic contaminants are: (1) the provision of stable and reproducible sensing chemistries to achieve the desired device performance and allow manufacture, and, (2) to rapidly process on-line statistically representative samples (e.g. litres of fluid or grams of tissue) and deliver isolated target molecules to the sensing device in a small volume aliquot. We have developed a robust and reliable optical sensor technology for nucleic acid analysis. In a project funded by Genome Canada, we are currently developing a high sensitivity detection system prototype that will be capable of measuring nucleic acid targets directly from samples (i.e. without the need for enrichment of the nucleic acid concentration by amplification methods such as, for example, PCR). We are therefore well poised to address the second key challenge of rapid sample preparation that will permit near real-time automated analysis of pathogens in foodstuffs. On-line cell capture, ultrasonic disruption and microfluidic methods will be developed for rapid isolation and ultra-concentration of marker nucleic acids from food washes and surface wipes, and water, which will permit automated analysis for critical pathogens using our new nucleic acid biosensor technologies. By developing this missing link in the overall diagnostic method, rapid quantitative testing for multiple targets including bacteria, viruses and parasites at each link in the food supply chain may be realised. The technology will provide for inexpensive (per test) reliable screening that can be used in abattoirs, food processing plants and in-field, as identified as goals of the HACCP program.

**Expected Impact of Project Outcomes on Food Safety in Ontario:**

It is estimated that over 80 million food-borne illnesses occur in North America each year, and that these can largely be attributed to *E. coli*, *Salmonella*, *Campylobacter* and *Listeria monocytogenes* (P.S. Mead et al., *Emerg. Infect. Dis.*, 5: 607, 1999). Since Walkerton there have been a number of *E. coli* outbreaks attributed to the food supply. Public health officials have been anxious for a rapid monitoring system to assess food and water supply systems for microbial contamination. We have developed optical sensors that could rapidly detect interfacial nucleic acid hybridization with high selectivity. Results showed that genotyping hybridization assays could be done in minutes and that selective binding was observed with discrimination of single-nucleotide polymorphisms. Measurements were complete in 1-2 minutes, and the sensors have been demonstrated to be sufficiently robust that they may be reused for over 500 cycles of application (months of use) with good reproducibility (C.V. < 15 %) and no indication of degradation. The ultimate goal is approximately 1000 determinations per hour, at detection levels of 1000 molecules of nucleic acid target, i.e. single cells or a few cells when screening RNA. Our advances in detection technology have made it even more important to shift focus towards creation of methods to decrease the amount of time and effort necessary for sample collection and preparation. The problem is to take a sample of some liters in volume or grams in tissue mass, selectively collect organisms of interest while eliminating most of the sample volume, and then to produce a measurable target and bring this target in a concentrated small volume to a detector array. In partnership with Genome Canada, and an industrial partner, SafeGuard Biosystems, we intend to develop biosensor technologies that have the potential to provide the speed, selectivity and sensitivity required for effective analysis of food and water.

## DM3: *Mycobacterium Avium* Subsp. Paratuberculosis: Novel Tools to Identify Risk for Contamination of Foods and Environments

### Abstract:

The goals of this research are to evaluate a three-step procedure to identify the presence of live *Mycobacterium avium* subspecies paratuberculosis (MAP) comprised of, i) affinity-based bacterial extraction, ii) broth enrichment and iii) mRNA-based identification, for the potential to improve sensitivity and reduce detection time of the contamination or infection. Current procedures utilize extensive sample processing and decontamination to separate the relatively few MAP from large numbers of microbial contaminants. Although the sensitivity of testing by culture on agar is accepted as poor, and takes 6-18 weeks for results, it is widely applied as the best method available to detect live MAP. At present there is no recommended standard laboratory protocol for live MAP tracking in milk or other sample types. Given this lack of standards for sample preparation, decontamination, and culture there is considerable variability in assay sensitivity, contamination rates and time-to-detection among laboratories when processing identical sample types.

Using various sample types, the study shall determine assay reliability, specificity, detection range, and cost. The study will examine the procedures for their application in clinical and food laboratories. Specifically, the study will focus on the following 3 components of the assay:

- a) Evaluate monoclonal antibody-based immunomagnetic capture for rapid and specific isolation of MAP cells.
- b) Evaluate the potential of broth enrichment to increase the sensitivity of the assay.
- c) Optimize an RNA-based protocol to detect MAP-specific transcripts, hence viable MAP, directly in the captured MAP or after periods of broth enrichment.

MAP is the cause of Johne's disease (JD), and affects approximately 37% of Ontario dairy herds. Feces, milk, blood and lymph nodes from infected animals can be contaminated with MAP bacteria. The infectious dose of MAP is not known.

Some MAP bacteria escape killing during pasteurization and present an exposure risk to susceptible humans. Currently, it is not known what factors affect MAP persistence in the environment, its survival in milk, or in meat. The potential for contamination of milk and meat within the Ontario dairy industry are not known.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

Through collaboration with the University of Guelph Laboratory Services Division the study will develop an assay for application in diagnostic service to test both specimens for disease and food products for contamination. The impetus for the development of a widely available, reliable, sensitive and rapid assay is that it will enable the following:

- a) Disease management: Reliable data are needed on the biological and on-farm environmental sources of live MAP. Informed strategies can then be implemented to reduce transmission within and among herds. There are no effective treatments or vaccines. Identifying and removing sub-clinically infected animals and other on-farm sources of infection will best achieve control of JD. Wild ruminant and non-ruminant species infected with MAP are a concern as they can act as reservoirs for the disease within a farm and as vectors carrying the disease between farms and so interfere with efforts to control the disease in livestock.
- b) Human Food Safety: Pasteurization studied over a wide range of temperatures and time combinations either report effective killing of MAP or tailing with live bacteria. It is apparent that live MAP is present in retail milk. Blood, lymph node tissue, feces and milk of animals with subclinical disease can contain MAP organisms. Concerns regarding the zoonotic potential of MAP indicate that the contamination of all products designated for human consumption be accurately assessed, then reduced or eliminated and monitored.

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## DM4: Reagentless Impedimetric Biosensors for Detection of Pathogens in Greenhouse Operations and Pork Processing

**Abstract:**

The time between the introduction of a biohazard and subsequent detection can strongly influence its impact (both in terms of health and economic). Although rapid detection methods are available these are typically laboratory based. Therefore, even if analysis can be performed within hours the time to collect, deliver and process the sample can take days. In this respect there is an identified need for sensors that can be used on-site that act as an alarm for the presence of potential biohazards. In the following project novel reagentless sensors for the detection of fecal indicators and pathogens will be developed.

The sensors are based on conducting polymer nano-tubes formed within microporous polycarbonate membranes onto which bioaffinity agents (host cell or antibodies) are immobilized. Interaction of the immobilized bioaffinity agent with analyte induces changes in the electrochemical properties of the conducting polymer film that can be detected using impedance spectroscopy. In the current project the target analytes selected will be F(+) coliphage (bacteriophage commonly linked to fecal contamination) and *Salmonella*. However, the generic sensing approach can be used to detect any biohazard (bacterial pathogens, enteric viruses, protozoa, endospores, toxins) through judicious choice of bioaffinity agent.

Initial work will fabricate the polymer electrodes and optimize immobilization of bioaffinity agents within the conducting polymer nano-tubes. The kinetics, sensitivity and selectivity of the sensors will be determined. Sensor performance will be validated against current standard techniques. Finally the ability to detect contamination within irrigation water or pork processing facilities using the sensor devices will be evaluated. Matrix effects on sensor performance will be evaluated and minimized through techniques such as background subtraction.

The sensors to be developed will be reagentless with no sample volume limitations or significant user input. The projected cost of the sensor strips will be \$2-5 and have a detection time of 5- 40 minutes. The limit of detection for coliphage will be in the order of 1 pfu/ml. The lower limit for *Salmonella* detection is projected to be 1-10<sup>2</sup> cfu/ml.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

OMAF has been pro-active at introducing HACCP and traceability schemes throughout the food chain. This in turn has led to an increase in end-product screening to reduce the risk of contaminated products reaching the marketplace. However, a more efficient approach would be to screen for hazards at critical points within the food chain thereby restricting the dissemination of contamination.

The sensors will be fabricated from relatively inexpensive materials and it is anticipated that each unit cost no more than \$2-5. By using plant derived antibodies (developed by Prof. C. A. Hall) the cost can be further reduced. Hand-held impedance analyzers are now commercially available and can be customized to the appropriate measurement type required.

Availability of robust, reliable and cheap biohazard sensors is the foundation of effective biosecurity, HACCP and traceability schemes. In this respect the generic sensing approach to be developed will be of direct benefit to food producers, processors, retailers and regulators.

# Risk Assessment

## RA1: A Novel Evidence-Based Tool in Support of Food Safety Policy Development

### Abstract:

Policy makers are increasingly dealing with complex food safety and public health issues such as BSE, antimicrobial resistance, avian influenza, food-borne and other emerging pathogens and hazards. The recently published 'Justice Haines Report' has strongly recommended the use of a science-based approach to food safety and more transparent food safety policy making in Ontario. In a world where information is only a click away, food safety professionals and policy makers need to identify information in a timely way, and to appraise and synthesize the best evidence on targeted issues. They also need to refine this evidence, to evaluate risks and to select optimal mitigation strategies within the Ontario context. Therefore, this research team, comprising researchers from two Canadian universities (Guelph, McMaster), two governmental agencies (Public Health Agency of Canada and OMAF) and collaborators from Iowa State University, proposes the development and evaluation of a novel evidence-based tool as a potential standard tool in support of food safety policy.

The tool will combine two existing knowledge synthesis and knowledge transfer methodologies. These are systematic review and risk assessment. Although they have been extensively used individually in other professional sectors, this is the first attempt to combine and adapt them to food safety policy making. The first methodology will enable trained research teams to identify, evaluate, rank and summarize, qualitatively and quantitatively, the best existing evidence on targeted issue(s). The second methodology will utilize this evidence and synthesize it into a characterization of the system so as to model risks associated with different scenarios/options. The tool will be evaluated using one relevant food safety and trade issue, namely '*Salmonella* in pork'. This issue was selected because of recent international and domestic trends that indicate an urgent need for policy development. However, it will be applicable to any food safety issue and agri-food sector in Ontario.

The project will be carried out in two phases. In phase 1, the research team will develop and evaluate the protocol for conducting systematic reviews on food safety effectiveness research. A rigorous and transparent systematic review will be conducted to evaluate and rank the effectiveness of potential on-farm interventions against *Salmonella* in swine and their estimated impacts on *Salmonella* reduction. In phase 2, quantitative risk assessment models will be developed to refine selected interventions, to evaluate risks throughout the pork chain and to select optimal mitigation strategies against *Salmonella* within the environment of the Ontario pork production system. A transparent, evidence-based summary and recommendations will be communicated to Ontario's policy makers and pork industry. The needs, gaps and opportunities for using systematic review and risk assessment as a potential standard tool in support of evidence-based food safety policy making will be evaluated.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

A novel evidence-based tool will be developed and evaluated as a potential standard tool in support of food safety policy making in Ontario. The resulting tool will support timely and informed food safety policy making in Ontario. Ontario's policy makers and agri-food sectors will negotiate existing and forthcoming food safety issues based on powerful and sound evidence. Baseline and other research data generated through previous OMAF-funded projects will be utilized in support of food safety policy making. The resulting information will allow the Ontario pork industry to appropriately evaluate interventions against *Salmonella* in pork and to make informed policy decisions regarding potential control options.

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## RA2: Prevalence and Enumeration of Foodborne Microbial Hazards in Retail Raw Meat Products in an Ontario Community

### Abstract:

To address food-borne illness in Ontario, the proposed study will link with a Public Health Agency of Canada/Agriculture and Agri-Food Canada (PHAC/AAFC) surveillance initiative that is measuring the prevalence of enteric pathogens in animals, retail food, water and humans. The study objectives include linking with the C-EnterNet project to facilitate the enumeration and additional sub-typing of retail food pathogen isolates for one year. In addition, a summary of human health enteric illness data will be developed, in conjunction with analysis of the retail food contamination levels.

The collaborative nature of this project, and the development of retail-level baseline enumeration data for the most important enteric food-borne pathogens, will contribute valuable Ontario-specific data for risk assessment activities and the development of Ontario's Food Safety Objectives. The study directly addresses a number of recommendations from the Haines report. Collaborations will involve the Region of Waterloo Public Health Unit, the Ontario Ministry of Health and Long Term Care, the Public Health Agency of Canada (Laboratory for Food-borne Zoonoses and Centre for Infectious Disease Prevention and Control), and Agriculture and Agri-Food Canada. Discussions are currently being pursued with the Centers for Disease Control (CDC) in the US to collaborate on a component of the retail raw meat study (specifically targeting *Campylobacter* levels on poultry products), and could provide some international comparison data.

The study design is a cross-sectional survey of raw retail pork, beef and poultry contamination (at the consumer level) for a 12-month period, to generate valid and representative data about consumer-level exposure to food-borne pathogens in meat, in one Ontario community.

The study area will be the Regional Municipality of Waterloo, a community of 470,000 residents with urban/rural demographics similar to the Canadian average.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

Science-based risk assessment along the food continuum is the modern approach to food safety regulation and policy development and is strongly endorsed by all levels of government. Unfortunately, there are limitations when risk assessment principles are applied to food safety issues, including the complexity of the food system, the lack of baseline data and inherent assumptions made due to information gaps.

This project is a collaborative initiative with a five-year PHAC/AAFC study, which will provide Ontario-specific baseline prevalence and enumeration data for enteric pathogen contamination of raw meat products, in order to provide relevant data that can be used in risk assessment.

This study will have many applications to current food issues. Pathogen enumeration baseline data will support OMAF risk managers in the prioritization of mitigation efforts, policy development, and the assessment of Ontario quality assurance and HACCP programs. As well, a number of Justice Haines' recommendations would be addressed.

C-EnterNet will integrate human, agri-food and water data, through enhanced typing procedures, to provide a comprehensive understanding of the link between food-borne pathogens and human health outcomes in Ontario. One of the study objectives is to foster collaborative efforts between jurisdictions and disciplines. One potential collaborative opportunity is a *Campylobacter* enumeration study on retail chicken, which is in development by the CDC in the US. These collaborations will provide an international perspective to the issue of retail meat contamination, and an exponential return on the investments of all of the collaborative partners.

# Risk Management and Control

## RM1: Control of *Salmonella* and Other Pathogens of Public Health Concern on Ontario Pig Farms

### Abstract:

We propose to conduct field studies on Ontario pig farms in order to establish effective intervention strategies to minimise the prevalence of pathogens of public health significance. In particular, we hope to demonstrate how *Yersinia enterocolitica* and *Salmonella* sp can be controlled. These two disease agents appear to be the most important food-borne pathogens associated with pork. If these organisms can be reduced at the farm level then the risk through the rest of the food chain will be also lessened.

Over the past 5 years, we have monitored approximately 80 finishing herds on an annual basis and have identified herds with a high prevalence to either *Yersinia* or *Salmonella*. In the case of *Yersinia*, a major risk factor appears to be environmental contamination and we hypothesised that strict hygiene and reduced mixing of pigs will greatly reduce the prevalence of *Yersinia enterocolitica*. On the other hand, *Salmonella* appears to be more closely associated with feeding techniques. We have shown that farms using fermented liquid-feeding are much less likely to have *Salmonella*-shedding market hogs compared to farms using dry-feeding. It has been suggested that the main reason for this apparent protection is the acidification of the diet. Whereas, liquid feeding is an expensive technology that tends to be only available to large farming operations, water acidification is easily applied to any size of pig farm, making it a more acceptable approach.

We propose to select 10 farms that we have identified as having a high prevalence of *Salmonella* and another 10 farms that have a high prevalence of *Yersinia*. Initially, we will investigate the spread of disease within each farm to determine when pigs appear to become infected and therefore determine at which stage of production intervention would be most appropriate. We will conduct field trials to evaluate intervention strategies on 5 farms with *Salmonella* and 5 farms with *Yersinia*.

Intervention on five *Salmonella* farms will consist of water medication with an acidifier. For five *Yersinia* farms, intervention will be improved hygiene and control of mixing of pigs. Ten control and ten treatment pens will be assigned and followed from entry in the grower-finisher barn until market on each farm. Culture of faeces of market age pigs will be used to determine the success of the treatment.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

Pork production is a major agricultural industry in Ontario and food safety has become an important issue with regard to ensuring both domestic and export markets. Countries, such as Denmark, have instituted on-farm monitoring programs to measure the prevalence of *Salmonella*. There will be pressure to institute similar programs in Ontario. The success of a monitoring program depends on whether or not farms that are identified with a high pathogen load can be instructed as to how they might be able to reduce the prevalence of *Salmonella* or other pathogens. At present, it is unlikely that some of the steps used in Denmark and elsewhere could be easily implemented or would be successful.

The Ontario pork industry is very diverse with many different farm types and management systems. In order to be able to implement a program to minimize pathogens of public health concern, intervention strategies need to be relatively inexpensive and practical for small farming operations as well as large multi-site co-operatives. The benefit of this research is to ensure that Ontario pig farmers are able to respond to demands and pressures in the area of food safety. Our goals are to develop the monitoring tools and practical intervention strategies to assist Ontario pig farmers to meet food safety standards of the future.

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### Collaborating Researcher(s):

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### Project Duration:

Jan. 2005 – Jan. 2007

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## RM2: Bacteriophage Therapy to Control *E. coli* O157:H7 in Cattle

**Abstract:**

Pre-harvest control of microbial food-borne and environmental hazards is an essential component of an effective food production system. Our earlier studies showed that bacteriophage (phage) therapy is potentially an effective intervention to control *E. coli* O157:H7 in cattle, which frequently carry this important human pathogen. The purpose of this project is to study the safety and efficacy of this therapy. We will study the safety of the phages in cattle and animals that share the same environments as cattle (e.g. rodents and birds) and that may be potentially exposed to the phages, and the efficacy of the therapy in cattle, firstly in controlled experimental infection studies in calves, and secondly in naturally infected cattle. This project will be conducted by researchers at the Laboratory for Foodborne Zoonoses of the Public Health Agency of Canada, Lethbridge Research Centre of Agriculture and Agri-Food Canada, and Nymox, a Canadian company, all of whom have considerable experience and interest in phage therapy. The outcomes of this project will provide information required for field trials and eventual transfer of this technology to field use.

**Expected Impact of Project Outcomes on Food Safety in Ontario:**

Recognising the impact of *E. coli* O157:H7 on marketability of cattle products domestically and internationally, cattle producers and processors in Ontario and elsewhere have embraced the need for on-farm control of this organism. Effective interventions at the farm level will not only enhance the microbial safety of cattle food products, but will reduce risks of contamination of water supplies, fresh produce, animals and the environment. Although several on-farm interventions have been investigated, most have met with little or only moderate success. However, we have shown “proof of concept” that treatment of cattle with *E. coli* O157 phages can potentially eliminate *E. coli* O157:H7 from cattle. This project addresses several remaining objectives of a larger project designed to further develop and transfer this technology to field use. If successful, this technology will benefit the Food Safety System in Ontario and the agri-food sector in general, through safer beef products, reduced contamination of the environment, water and other foods with *E. coli* O157:H7, reduced risk of human exposure to *E. coli* O157:H7, and enhanced market competitiveness in beef products domestically and internationally.

## RM3: Reduction of *Campylobacter jejuni* Colonization in Poultry

### Abstract:

*Campylobacter jejuni* is the leading cause of bacterial foodborne illness in North America. Contaminated poultry are the primary risk factor for *C. jejuni* infection in humans. Thus, reducing the levels of *C. jejuni* colonization in poultry is a priority in the area of risk management and control to increase food safety.

Supplementation of poultry feed with the high-efficiency feed supplement, xylanase, was demonstrated to cause changes in mucin carbohydrates and reduce mucin viscosity and *C. jejuni* colonization. In order to further exploit this finding, we will investigate the role of chicken mucin viscosity and carbohydrate content on *C. jejuni* virulence and colonization potential. In addition, NRC-IBS has engineered a superior xylanase supplement ([http://ibs-isb.nrc-cnrc.gc.ca/ibs/ourstories/iogenstory\\_e.html](http://ibs-isb.nrc-cnrc.gc.ca/ibs/ourstories/iogenstory_e.html)) that is able to resist extreme temperatures during animal feed pelleting and remains active in the chicken gastrointestinal tract. Together we will compare the effects of commercial and NRC-IBS modified xylanase on *C. jejuni* colonization with the intent to develop a cost-effective high efficiency animal feed supplement that will also improve Ontario Food Safety by reducing the levels of *C. jejuni* colonization in poultry. This objective will be achieved through the efforts of our group with internationally recognized expertise in the analyses of colonization factors (Szymanski/Allan), development of animal models (Allan/Bihun), carbohydrate structure elucidation (Brisson/Kelly), gene expression profiling (Nash) and xylanase engineering (Sung). These studies will involve using well developed in vivo model systems, state-of-the art analytical equipment available at NRC-IBS ([http://ibs-isb.nrc-cnrc.gc.ca/ibs/immunochemistry/bioanalysis\\_e.html](http://ibs-isb.nrc-cnrc.gc.ca/ibs/immunochemistry/bioanalysis_e.html)) and in-house developed *C. jejuni* microarrays ([http://ibs-isb.nrc-cnrc.gc.ca/ibs/immunochemistry/campychips\\_e.html](http://ibs-isb.nrc-cnrc.gc.ca/ibs/immunochemistry/campychips_e.html)).

### Expected Impact of Project Outcomes on Food Safety in Ontario:

*C. jejuni* is the leading cause of gastroenteritis in Canada resulting in a significant health burden to our economy. The primary risk factor for *campylobacter* infection is contaminated poultry. Recently it has been demonstrated that supplementation of chicken feed with xylanase causes a decrease in *C. jejuni* colonization with a simultaneous change in mucin viscosity and carbohydrate content. Other studies have demonstrated that both the physical changes in viscosity and the presence of mucin have affects on *C. jejuni* infectivity (2,3), Bourke, personal communication). We will further extend these observations by using our expertise in carbohydrates and understanding of *C. jejuni* regulatory pathways, to examine the xylanase induced changes in mucin and how this influences *campylobacter* colonization of poultry. In addition, chicken feed will be supplemented with commercial xylanase and compared with an NRC-IBS modified xylanase to determine reduction of *campylobacter* colonization. With Iogen Corporation (Ottawa), the NRC-IBS enzyme has been engineered to resist extreme temperatures during animal feed pelleting, remains active in the chicken gastrointestinal tract, and facilitates efficient feed conversion through better digestion and assimilation, leading to enhanced meat and egg production. Thus, the modified xylanases, currently approved for use in pulp bleaching with annual sales in the millions, have further applications in food safety and the livestock industry.

### Project Leader:

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Pathogen Genomics

### Collaborating Researcher(s):

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### Project Duration:

Jan. 2005 – Dec. 2006

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## RM4: Ultra Violet and Hydrogen Peroxide Combination for Decontaminating Minimally Processed Fruits and Vegetables

### Abstract:

Minimally processed vegetables and fruits are become established as a significant vehicle for foodborne pathogens. Due to the open nature of the fresh produce chain, contamination of the product can occur at any point during cultivation and post-harvest handling. Because bacteria (including human pathogens) can become located within sub-surface structures of plants (stomata, cut-edges) simply washing produce is inadequate even when chemical sanitizers such as hypochlorite are used.

In this study the efficacy of applying a combination of Ultra Violet (UV)/hydrogen peroxide ( $H_2O_2$ ) in decontaminating produce will be evaluated. When  $H_2O_2$  (<2% v/v) is illuminated with UV light highly anti-microbial, but short-lived, hydroxyl radicals are formed. This provides a greater kill effect than when either UV or hydrogen peroxide is applied alone. Although the combination of UV and hydrogen peroxide have been used for over 20 years for sterilizing carton packaging it has not been fully evaluated as a method for decontaminating fruit or salad vegetables.

In the proposed project a treatment chamber will be constructed with the capacity to decontaminate 3 kg batches of produce. Optimization of operating conditions will be performed to maximize the generation of hydroxy radicals from  $H_2O_2$  (0.2-1%). The kinetics and mode by which UV/  $H_2O_2$  inactivates a range of pathogenic (*E. coli* O157, *Salmonella*, *L. monocytogenes*, *Aeromonas hydrophilia*) and spoilage (*Pseudomonas fluorescens* and *Erwinia carotovora*) bacteria, in addition to MS2 coliphage (enteric virus surrogate), will be undertaken. The efficacy by which the treatment can decontaminate a diverse range of product types (lettuce, red cabbage, spring mix, spinach, red onion, carrot, tomatoes, cantaloupes, strawberries, raspberries and apples) will be evaluated. A 5-log reduction in bacteria/bacteriophage numbers without adversely affecting product quality will be used as the criteria for success.

The project brings together Elopak (specialists in UV:  $H_2O_2$  decontamination technology), Pride Pak Salads (major fresh cut producer within Ontario) food microbiologists and engineers.

### Expected Impact of Project Outcomes on Food Safety in Ontario:

The fresh-cut industry represents a significant sector of the Ontario economy with an estimated Farm Gate Value of \$224 M per annum. Although foodborne outbreaks linked to fruit and vegetables are rare within Ontario there is a need to take a pro-active approach with respect to ensuring high food safety standards within the industry.

Currently there is no intervention step within the fresh cut chain that can ensure the removal of field acquired contamination. It is widely acknowledged that aqueous wash-based systems have limited ability to effectively penetrate pathogens present in sub-surface locations. The proposed approach of using UV/  $H_2O_2$  is specifically aimed at inactivating pathogens located in such protective sites. The method is based on generating an intense burst of anti-microbial hydroxyl radicals that rapidly inactivate microbes located on the surface and sub-surface of produce. Because of the transient nature of the radicals it is envisaged no bleaching of the product will occur.

The project will provide a viable, cost effective, alternative to current produce decontamination methods. If successful, the UV/  $H_2O_2$  based decontamination system will significantly enhance the safety of minimally processed fruit and vegetables. The method will also find utility in other sectors especially in relation to meat processing where virulent pathogens such as *E. coli* O157 are an obvious concern.

# SECTION THREE: Status of previously funded projects (2000/01–2003/04)

The details about previously funded projects by the program are available on our website:  
[www.omaf.gov.on.ca](http://www.omaf.gov.on.ca)

Lead Researcher	Project Title	Status
<b>Detection Methodology (DM)</b>		
Dr. Archambault Marie, University of Guelph	Johne's Disease – New Test Validation	Completed
Dr. Bidawid Sabah, Health Canada, Ottawa	Development of a Rapid Microarray Diagnostic Assay for Detection of Norwalk-like Viruses in Food	Ongoing
Dr. Brown Stephen, Queens University	Development of a Rapid, Sensitive and Reliable Test for the Detection and Quantification of <i>Escherichia coli</i> in Foods	Completed
Dr. Chen Shu, University of Guelph	Improvement and Validation of a DNA Microchip-based Test for Rapid and Simultaneous Detection of Six Food-borne Pathogens in Food Samples	Completed
Dr. Chen Shu, University of Guelph	Development of a Novel Protein Chip-based Test for Rapid and Cost-effective <i>Salmonella</i> Serotyping	Completed
Dr. Chen Shu, University of Guelph	Development of a Robust DNA Preparation Method to Enhance Simultaneous Detection of Multiple Pathogens in Foods by a Microarray-based Assay	Ongoing
Dr. Griffiths Mansel, University of Guelph	New Technologies for Improving Real-time PCR Methods for Detection of Food-borne Pathogens	Completed
Dr. Griffiths Mansel, University of Guelph	Rapid Phage-based Method for the Detection of Pathogens in Food	Ongoing
Dr. Gyles Carlton, University of Guelph	Validation of a Method for Determining the Species of Origin of Contaminant <i>E. coli</i>	Completed
Dr. Hall Christopher, University of Guelph	Fluorescence Polarization Immunoassays (FPIA) for Food Safety: a Rapid Detection System for Pathogens and Chemicals	Ongoing
Dr. Ismail A.A., McGill University	Rapid Whole-Organism Identification Methods Based on Fourier Transform Infrared (FTIR) Spectroscopy	Ongoing
Dr. McEwen Scott, University of Guelph	<i>Campylobacter</i> Isolation Methodology and Molecular Characterization	Completed
Mitchell Mark, University of Guelph	Development and Validation of a Screening Protocol for Identifying Sulfamethazine-violative Swine Carcasses at Ontario Abattoirs Using the CHARM ROSA™ Sulfamethazine Test	Completed
Dr. Odumeru Joseph, University of Guelph	Application of the Impedance and Colorimetric Systems for Rapid and Cost Effective Detection of <i>Listeria</i> Species in Food and Environmental Samples	Completed
Dr. Odumeru Joseph, University of Guelph	Evaluation of Immunoassay Based Kits with High Sensitivity and Specificity for Rapid Detection of <i>E. coli</i> O157:H7 in Foods	Completed
Dr. Schraft Heidi, Lakehead University	Detection of <i>Campylobacter jejuni</i> by Fluorescent <i>in Situ</i> Hybridization	Completed
Spilsbury Louise, University of Guelph	Development of an Analytical Method for the Confirmation of Sulfonamides in Animal Tissues	Completed

Lead Researcher	Project Title	Status
<b>Risk Assessment (RA)</b>		
Dr. Allan Brenda, University of Saskatchewan	Identification of Bacterial Components that Influence Colonization of Poultry by <i>Campylobacter jejuni</i>	Completed
Dr. De Lange, C. F. M., University of Guelph	Liquid Feeding of Swine – Potential Positive and Negative Impacts on Pork Safety	Completed
Dr. Friendship Robert, University of Guelph	Surveillance of Ontario Pig Farms for Diseases of Public Health Significance	Completed
Dr. Griffiths Mansel, University of Guelph	Investigation of Routes for Transfer of Food and Water-borne Pathogens to Produce	Completed
Dr. Holley Richard, University of Manitoba	Evaluation of Pesticide Solutions in the Transmission of Pathogenic Bacteria to Horticultural Crops	Completed
Dr. Kelton David, University of Guelph	Assessing the Incidence of Antimicrobial Resistant <i>E. coli</i> and <i>Salmonella</i> Bacterial Isolates in Cull Cows from Ontario Free Stall Dairy Herds	Completed
Dr. McEwen Scott, University of Guelph	Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Poultry Products and Human Cases in a Southwestern Ontario County	Completed
Dr. Ribble Carl, University of Guelph	Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Turkey and Veal Products in Southwestern Ontario	Completed
Dr. Warriner Keith, University of Guelph	Establishment of Critical Control Points for Enteric Pathogens in Beef Production	Ongoing
<b>Risk Management</b>		
Dr. Abernathy, Tom McMaster University	A Community Trial to Determine an Effective Intervention for the Delivery of HACCP to the Food Service Sector	Completed
Dr. Gong Joshua, Agriculture and Agri-Food Canada, Guelph	Evaluation of Essential Oils as an Alternative to Dietary Antibiotics to Control Food-borne Pathogens in Livestock	Ongoing
Dr. Henson Spencer, University of Guelph	Understanding Barriers to the Effective Implementation of HACCP in the Ontario Food Processing Sector	Completed
Dr. McAllister T. A. , Agriculture and Agri-Food Canada, Lethbridge	Evaluation of the Ability of Seaweed Extract (Tasco-14) to Reduce the Duration and Intensity of Fecal Shedding of <i>Escherichia coli</i> O157:H7 and Total <i>E. coli</i> by Cattle	Ongoing
Dr. Sharif Shayan, University of Guelph	Antibiotic Replacement Therapy for Control of Food-borne Pathogens in Poultry	Ongoing
Skinner Alison, Ontario Beekeepers' Association	Enhancing the Food Safety of Honey Bee Hive Products through the Use of Organic Beekeeping Practices and Effective Monitoring of Pest and Disease	Completed
Dr. Warriner Keith, University of Guelph	Elimination of Human Pathogens on Seeds Destined for Sprout Production Using a Novel Sanitizer	Completed
Dr. Zhou H., University of Guelph	Practical Optimization of Ozonation Process for Enhancing Microbial Safety and Food Quality	Completed

# SECTION FOUR: QUICK SEARCH

<b>Subject:</b>	<b>Abstract:</b>	<b>Subject:</b>	<b>Abstract:</b>
Animal feed supplement	RM-3	Novel, evidence-based tool	RA-1
Bacteriophage therapy	RM-2	On-farm control	RM-2
Baseline study	RA-2	On-line detection	DM-2; DM-4
Beef	RM-2	Pig farms	RM-1
Biosensors	DM-2; DM-4	Poultry	RM-3
<i>Campylobacter</i>	RA-2; RM-3	Pork	RA-1; RM-1
Coliphage	DM-4	Protein-based microarray	DM-1
Dairy	DM-3	Risk assessment	RA-2
<i>E. coli</i> 0157:H7	RA-2; RM-2; RM-4	<i>Salmonella</i>	DM-1; DM-4; RA-1; RA-2; RM-1; RM-4
Food safety policy	RA-1	Salmonella serotyping	DM-1
Fruits and vegetables	RM-4	Sample concentration	DM-2
Haines report	RA-1	Sanitizer	RM-4
Johne's disease	DM-3	Spoilage bacteria	RM-4
<i>Listeria monocytogenes</i>	RM-4	Systematic review	RA-1
Live MAP	DM-3	UV/Hydrogen Peroxide	RM-4
Meat	DM-3; RA-2	Water acidification	RM-1
Microarray	DM-1	Water irrigation	DM-4
Milk	DM-3	Xylanase	RM-3
<i>Mycobacterium paratuberculosis</i>	DM-3; RA-1	<i>Yersinia</i>	RM-1





