Université de Montréal

ASSOCIATION BETWEEN PATHOGENIC AND INDICATOR BACTERIA AND SOME CONTROL POINTS OF A RISK ASSESSMENT MODEL FOR FOOD PRODUCING ESTABLISHMENTS IN QUEBEC, CANADA

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RÉSUMÉ

L'atténuation du risque d'agents pathogènes d'origine alimentaire dans les établissements de transformation des aliments est un élément essentiel du système de contrôle de la sécurité sanitaire des aliments d'un établissement ou d'une perspective de l'agence de contrôle des aliments. L'objectif de cette étude était d'estimer l'association entre les microorganismes sélectionnés et les 20 points de contrôle d'un modèle d'inspection d'évaluation du risque. Une étude transversale a été menée sur un échantillon pratique de 18 établissements de restauration de viande prête à manger (RTE) situés au Québec entre juin et juillet 2015. Des écouvillons ont été utilisés pour échantillonner des surfaces de 900 cm^2 par établissement ; trois surfaces avec contact alimentaire et deux sans contact avec des aliments. La PCR en temps réel a été réalisée en utilisant SYBER Green, ciblant le gène siiA de *Salmonella* et les gènes prfA et prs de *Listeria monocytogenes*. Nous avons détecté un isolat de *Salmonella* (1%) et 18 de *L. monocytogenes* (20%) parmi les 90 échantillons prélevés dans tous les établissements. *E. coli* était présent dans 7 des 18 établissements (39%). La valeur moyenne du Log10 de *E. coli* et le compte des aérobies totaux (TAC) étaient de 1,16 et 5,01, respectivement. Parmi les points de contrôle évalués par les inspecteurs, la "température ambiante" et la présence de "facteurs potentiels favorisant l'introduction de microbes pathogènes, contaminants toxiques (dangereux)" ont démontré une relation positive avec le niveau de *L. monocytogenes* (p = 0,03) et (p = 0,04) respectivement. *E. coli* a également été positivement associé aux points de contrôle "température ambiante" (p = 0,02) et "sources environnementales de contamination" (p = 0,005). La « présence d'installations adéquates de lavage des mains » était liée à la charge de TAC (p = 0,01) dans les établissements. Cette étude fournit un nouvel aperçu de la relation

entre l'évaluation des points de contrôle spécifiques faite par un inspecteur et la charge microbiologique dans les établissements alimentaires.

Mots-clés : établissements alimentaires, agents pathogènes d'origine alimentaire, modèle d'inspection, points de contrôle, Culture, RTi-PCR, Québec

ABSTRACT

Mitigating the risk of foodborne pathogens in food processing establishments is an essential part of food safety control system from an establishment or a food control agency perspective. The objective of this study was to estimate the association between selected microorganisms and scores from 20 control points of a risk assessment inspection model. A cross-sectional study was conducted on a convenient sample of 18 meat processing ready to eat (RTE) establishments food establishments located in Quebec between June and July 2015. Sponge swabs of 900 cm^2 in surface area of three food contact and two non-food contact surfaces were sampled per establishment. *Real Time-PCR* was done using SYBER Green, targeting the *siiA* gene of *Salmonella* and both *prfA* and *prs* genes of *Listeria monocytogenes*. We detected one *Salmonella (1 %)* and 18 *L. monocytogenes (20 %)* isolates from the 90 samples collected in all establishments. *E. coli* was present in 7 of 18 (39%) establishments. The mean Log10 counts of *E. coli* and Total Aerobic Count (TAC) were 1.16 and 5.01, respectively. Among control points assessed by inspectors, "*ambient temperature*" and the presence of "*potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants*" showed a positive relationship with the level of *L. monocytogenes* (p = 0.03) and *(p= 0.04)* respectively. *E. coli* was also positively associated with the control point "*ambient temperature*" *(p* =0.02) and "*environmental sources of contamination*" (p = 0.005). The "*presence of adequate hand washing facilities*" was linked to the load of TAC (p = *0.01*) in the establishments. This study provides new insight on the relationship

between inspector assessment of specific control points and microbiological load in food establishments.

Key words: *Food establishments, Foodborne pathogens, inspection model, control points, Culture, RTi-PCR, Québec*

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LIST OF ABBREVIATIONS

APC: Aerobic Plate Count

AAFC: Agriculture and Agri-Food Canada

CFIA: Canadian Food Inspection Agency

CFU: Colony Forming Unit

EPEC: Enteropathogenic *E. coli*

FCS: Food Contact Surfaces

FDA: Food and Drug Administration

FSMS: Food Safety Management System

FSIS: Food Safety and Inspection Services

GMP: Good Manufacturing Practices

HACCP: Hazard Analysis and Critical Control Points

HC: Health Canada.

IR: Inherent (initial) risk

MAB : Mesophilic Aerobic Bacteria

MAPAQ : Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

NFCS: Non-food Contact Surfaces

OIR: Official Inspection Report

PHAC: Public Health Agency of Canada

PCR: Polymerase Chain Reaction

QMRA: Quantitative Microbial Risk Assessment

RTi-PCR: Real time Polymerase Chain Reaction

SPC: Standard Plate Count

TAC: Total Aerobic Count

USDA: United States Department of Agriculture

*VTEC: V*erotoxigenic *E. coli*

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CHAPTER 1. INTRODUCTION

Consumption of food products or beverage contaminated by disease-causing bacteria, parasite, virus or chemical compounds can result in gastrointestinal disturbance and a condition referred as foodborne diseases or more commonly food poisoning *(31, 89)*. Food can be contaminated at different points in the food production and preparation processes. Globally, increase in the occurrence of foodborne illnesses continue to be a concern at the international level *(151)*. Worldwide, foodborne and waterborne diarrheal diseases together kill about 2.2 million people yearly *(155)*.

According to the WHO, about 600 million cases of illness were recorded in 2010 and attributed to 31 foodborne hazards. Diarrheal diseases caused by infectious agents represented approximately 550 million of the cases, of which norovirus (120 million cases) and *Campylobacter* spp. (96 million cases) were the primary bacterial pathogens. In addition, hepatitis A virus (14 million cases), the helminth Ascaris spp. (12 million cases) and the typhoid bacterium *Salmonella* Typhi (7.6 million cases) were also common causes of foodborne illness. Foodborne diarrheal disease agents also caused 230,000 of the 420,000 deaths due to foodborne hazards. Of these, non-typhoidal *S. enterica* accounted for 59,000, enteropathogenic *E. coli* (EPEC) for 37,000, norovirus for 35, 000, and enterotoxigenic *E. coli* (ETEC) for 26,000 deaths. Among 59,000 deaths due to non-typhoidal *S. enterica*, 32,000 were experienced in the two African sub-regions, and included 22,000 deaths due to invasive disease by this bacterium. The major non-diarrheal causes of foodborne deaths were due to *Salmonella* Typhi (52,000), the helminth Taenia solium (28,000), hepatitis A virus (28,000) and aflatoxin with 20,000 *(47, 132)*. Various reports indicated that foodborne illnesses result in serious economic losses.

Movement of people in processing establishments and the relative lack of strategies to regulate such activities increase the probability of introduction the pathogens like

Salmonella and *Listeria monocytogenes* at different stages of the production chain before consumption of ready to eat (RTE) food items. Occasionally, higher concentrations of *L. monocytogenes* may be present in RTE products that are both prepackaged and packaged in the store. The prevalence of *L. monocytogenes* may increase due to variation in the adsorption rate, bacterial serotypes, environmental condition, type of surface and pretreatment used. Indeed, *Listeria monocytogenes* adheres to the inert surfaces encountered in the food-processing environments *(130)*.

The use of *E. coli* as an indicator of fecal contamination started a century ago. This was based on the premise that *E. coli* is abundant in human and animal feces and not usually found in other ecological niches. Furthermore, since *E. coli* could be easily detected by its ability to ferment glucose (later changed to lactose), it was easier to isolate than other known gastrointestinal pathogens. Hence, the presence of *E. coli* in food or water became accepted as indicative of recent fecal contamination and the possible presence of frank pathogens *(139)*.

Application of risk analysis has improved food safety and was proven to be important in the development of food safety standards, design and implement tailored interventions, and to monitor the outcomes (both successful and unsuccessful) of these interventions *(39)*. Risk-based inspection starts with the consideration of hazards associated with the food and assess the sufficiency of control measures used *(41)*. Application of risk assessment has significant value in evaluating and managing foodborne microbiological health risks *(152)*. The microbial risk assessment (MRA) gives an approximation of the extent of human health risk in terms of likelihood of exposure to a pathogenic microorganism in food and the likelihood and impact of any adverse health effects after exposure. Furthermore, a detailed MRA can be used to realize the limitations

of activities and identify important gaps in our knowledge, characterize the most important risk factors in the farm-to-fork chain, help to design new strategies for risk mitigation, and provide guidance for determining priorities in public health and food safety research programs *(68)*. Quantitative microbial risk assessment (QMRA) is an approach used to identify various risk factors that influence food safety and ultimately provides an estimate of the level of illness that a pathogen can cause in each population *(75)*. Depending on the emphasis and the perspective of the exposure (risk) assessment, different approaches have been used in developing the overall risk analysis model *(34)*. Foodborne related hazards including microbial pathogens are controlled by the application of control measures in the food chain in a farm to table approach *(25)*. Currently, either there is no standard metric used to manage the entire food safety system or platforms used to collect food safety performance data on a global scale *(150)*. However, different countries used different food safety control system ultimately aimed to reduce foodborne hazards and provision of safe food to the public. For instance, the concept of measuring food production chain in Belgium is entirely dependent of the 'Status' of 30 food safety indicators during inspection which are indexed and used in establishing a safety barometer *(62)*. Given the difference in the production and processing of food, there are flexibility and changes in selecting control points at which validation of such control points is crucial *(25)*. Application of microbiological criteria is a tool used to guide hygienic production of food but not guarantee the safety of foodstuff tested *(35)*. Identifying microorganisms within the environment of food processing facility has an importance in preventing the pathogen from entering the food and contaminating food contact surfaces. This can be used as a systematic mechanism that gives valuable information in addressing prediction and early warning of foodborne pathogens *(135)*.

The combined application of inspection and microbiological testing has been indicated as a successful strategy to assess hygienic status of food safety control points, where microbiological criteria provide strategy and reference points for both food establishments in the process of managing the product and authorities to monitor foodstuffs. Whereas selected control measures are capable, on a consistent basis, of achieving the intended level of hazard control *(25, 59)*. Since the MAPAQ food inspection model has been implemented for several years, we found important to assess the control points and their association with microorganisms so that it will give an updated information in assessing the relative importance of these various inspection activities.

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CHAPTER 2. LITERATURE REVIEW

2.1. Foodborne Diseases

Foodborne diseases are getting a serious attention in many countries mainly because of the public health threat and economic importance *(112)*. In Canada, annually 1.6 million and 2.4 million cases of foodborne illness are associated with 30 known pathogens and unspecified causes respectively, resulting in a total estimate of 4.0 million cases of foodborne illness. Of these, there are about 11,600 hospitalizations and 238 deaths and the main pathogens responsible for these consequences are norovirus, nontyphoidal *Salmonella* spp., *Campylobacter* spp., VTEC and *Listeria monocytogenes* *(107, 132)*.

Listeria monocytogenes and *Salmonella* are among the different pathogens mentioned by the Public Health Agency of Canada (PHAC) and Canadian Food Inspection Agency (CFIA) as the common pathogens associated with food and waterborne illnesses *(15, 104)*.

Majority of cases related with foodborne diseases in Canada arise from an individual consumer's improper food handling, cooking and storage practice of consumers *(19)*. However, commercial sources of food have the potential to cause a significant illnesses *(19)*. Rapid expansion of international food trade, large scale farming, extensive food production and processing and complexity of the supply chains take part in favoring the occurrence of microbiological food safety outbreaks *(19, 74, 102)*. Despite the presence of multiple focal points that create an opportunity for contamination in the food supply chain, most food-borne illnesses can be prevented during the final preparation and handling of food *(88)*. The principle of pre-harvest food safety management at the farm level helps in improving animal health and performance to minimize economic losses due to disease. However, in intensive industrial production, the use of antimicrobials as a growth promoter

has promoted the emergence of antimicrobial resistance in enteric pathogens of food animals *(114, 126).*

2.2. Economic Cost of Foodborne Illness

To formulate and apply different strategies and decisions in the food safety system, knowing the estimates of both the incidence of foodborne illness and its financial impact are very important. Determining the extent of the problem also helps in assessing the effectiveness of any changes to food safety standards and regulations *(1)*. The selection or adequate methodologies in an attempt to estimate the economic burden of foodborne disease remains a challenge *(14)*. Based on reports that include a survey data, laboratory findings and reported foodborne diseases, each year millions of cases occur in Canada and USA. For instance, in 1989 in Canada, it was estimated about $1.1 billion cost was as a result of 1 million cases of acute bacterial foodborne illness *(133)*. Currently, the cost of foodborne illnesses in Canada is estimated based on two methods that consider cases issued from known pathogens for which epidemiological data are recorded and pathogens that are not part of standard surveillance. Under reported and under-diagnosed cases are also used during cost estimation by applying a special algorithm *(105)*.

About 5.5 million cases of foodborne origin cost about $7 billion back in 1989 in the U.S annually. Deaths, especially due to listeriosis, salmonellosis, Vibrio infections, and hemorrhagic colitis were a major contributor to the overall costs. Since it affects all parts of the food system, salmonellosis was the economically most important diseases and difficult to control by public health authorities and the food industry *(133)*. Periodically,

the Economic Research Service (ERS) of the United States Department of Agriculture (USDA) has been updating and expanding these analyses using better data and estimation method. Improved and detailed information on disease incidence, health outcome due to foodborne illness and methods used are major components to be incorporated in each estimation. Past evaluations were based on limited information about the incidence of foodborne illness and used the cost of illness(COI) method to calculate expenditures on medical care and lost productivity due to premature death and nonfatal illness *(14)*. The ERS estimation indicated an annual cost of foodborne illnesses in the U.S. exceeds $15.6 billion. More than 95 percent of foodborne-related illnesses were as a result of the combined effects of 15 major pathogens. The most expensive pathogen related to cases of foodborne illness was *Salmonella*, with treatment costs estimated at $3.6 billion. *Listeria monocytogenes* ($2.8 billion) and *Escherichia coli* ($271 million) are also other pathogens affecting the economy *(149)*.

2.3. Salmonellosis and Listeriosis

From an expert elicitation process that was conducted in Canada, major transmission routes (foodborne, waterborne, animal contact, person-to-person, and other) of 28 pathogens were estimated at the point of consumption and *Listeria monocytogenes* and *Salmonella* spp., were estimated as mostly foodborne *(13)*.

2.3.1. Salmonellosis

2.3.1.1. Etiology

Salmonella is a genus of the family *Enterobacteriaceae* and comprises a large and closely related population of medically important pathogens *(123)* and commonly found in the intestines of animals and birds *(106)*.

The two species are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* has six subspecies which are known by a Roman number and a name i.e. I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*. *S. enterica* subspecies are differentiated biochemically and by genomic relatedness *(10, 43, 100)*.

2.3.1.2. Epidemiology and Transmission

There is a worldwide occurrence of salmonellosis due to *Salmonella enterica*. However, prevalence varies from place to place *(51)*. *Salmonella enterica* serovar Typhimurium is the cause of non-typhoid salmonellosis, the diseases that is more common in developed countries whereas enteric fever is mostly found in Asia and other developing countries. Both *Salmonella enterica* Typhimurium and Enteritidis are known their zoonotic transmission *(51, 81, 101, 156)*.

People can become ill with salmonellosis due to ingestion of food contaminated with animal feces. Foods of animal origin are commonly contaminated as compared to fruits and vegetables *(106)*. In addition to foodborne transmission of salmonellosis, which is the principal means, direct or indirect animal contact are also causes of the disease. Apparently healthy animals and those clinically sick shed *Salmonella* for long periods of time. However, the latter group of animals is associated with higher prevalence of shedding *(52, 156, 157)*.

According to the OIE report, occurrence of Salmonellosis is highly dependent with the husbandry practices in which the diseases frequently occur in animals reared intensively. It is also reported that some serovars are host specific like S. Abortusovis in sheep, S. Typhi in humans or host adapted like S. Choleraesuis in pigs and S. Dublin in cattle *(157)*.

2.3.1.3. Public health importance

In Canada, yearly estimate of illnesses due to non-typhoidal *Salmonella* alone are 87,500 or 5% of the total illness and the number of hospitalizations and death (mortality) due to this pathogen are 925 (24%) and 17 (16%) respectively *(107, 131)*. The report from CDC in 2011 showed, eight major pathogens responsible for foodborne related diseases conditions and death and deaths. Among which, non-typhoidal *Salmonella* results in 1,027,561 illness, 19,336 hospitalization and 378 deaths in the USA *(136)*.

In 2012 there were 106 (25%) outbreaks and 3,366 (33%) illnesses in USA due to *Salmonella.* Among the 101 confirmed *Salmonella* outbreaks, Enteritidis was the most common serotype reported (26 outbreaks, 26%), followed by Typhimurium (13, 13%), Newport (10, 10%), Javiana (7, 7%), and Heidelberg (6, 6%) *(137)*. Depending on the serotype pathogenicity, humans might develop an invasive life-threatening or self-limiting gastroenteritis form of the disease. However, immunocompromised individuals, elders and young might develop severe form from the latter case *(153)*.

2.3.2. Listeriosis

2.3.2.1. Etiology

Human listeriosis is commonly caused by *Listeria monocytogenes, which* is a genus of Gram-positive bacteria. Under the genus, there are five listeria species, namely *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi. L. ivanovii*is is pathogenic for other mammals. *L. monocytogenes* has been largely studied in the past decades because of its importance as a food-borne human pathogen *(80, 90)*.

2.3.2.2. Epidemiology and transmission

Foodborne transmission of *Listeria* was conclusively demonstrated in 1981 in a Maritime Province of Canada, which involved contaminated coleslaw, as one of the first outbreak *(118)*. *L. monocytogenes* has been involved in numerous major outbreaks in the United States, Canada, Switzerland, Austria, France, England, and Wales during the past 20 years *(119)*. Human infections usually arise form ingestion of food that has linked with farm animals and their environment contamination. The genus *Listeria* are ubiquitous in nature and samples from different environmental sites including wastes from food producing establishments, slaughterhouses and abattoirs, meat, soil, vegetation, sewage, water, animal feed, and the feces of healthy animals reveal the presence of *Listeria (60)*.

2.3.2.3. Public health importance

Each year, in Canada, 35 (33%) of known causes of foodborne deaths is caused by *L. monocytogenes (107)*. This pathogen is known to cause self-limited febrile gastroenteritis in previously healthy individuals who ingest high numbers of it. However, *Listeria* can be fatal due to sepsis or central nervous system infection (meningitis or meningoencephalitis) in elderly adults, pregnant women, neonates and immunocompromised patients *(12, 119)*.

2.4. Hygiene Indicator Organisms

Food safety and the microbiological quality are usually y assessed by testing various indicator microorganisms which passed rigorous scientific evaluation and validation *(12)*. The presence of "indicator organisms" or "hygiene marker organisms" indicates a compromised Good Manufacturing Practices (GMP) which results in a food product of unacceptable microbiological quality. However, the "index", "marker", "simulator", or "surrogate" organisms are the one used as indirect tests that tell possible occurrence of pathogen *(61, 147)*. The presence of *E. coli* O157:H7 or *Salmonella* are usually assessed by indicator organisms, such as coliforms, thermo-tolerant coliforms, generic *E. coli*, *enterococci*, and *Enterobacteriacea*e *(147)*.

2.4.1. Escherichia coli

Escherichia coli is commonly found in the gastrointestinal tract (GIT) of humans and warm-blooded animals. It`s considered as harmless commensal that constitute about 1 percent of the normal gut microbial population and is the choice of indicator bacteria to detect and measure fecal contamination in the assessment of food and water safety mainly due it it`s abundance in the gut *(42)*.

The presence of *E. coli* in ready-to-eat (RTE) foods indicates fecal contamination and may be related to a lack in effectiveness of sanitation programs or/and inadequate heat treatment *(12, 129)*. A minimal contamination of some food products is however often unavoidable. For instance, the acceptable presence of indicator *E. coli* in RTE food is between <10 CFU/g <100 CFU/g which are the satisfactory and marginal values respectively. Counts ≥100 CFU/g are unsatisfactory and considered to be linked with increased likelihood of contamination by verotoxigenic or Shiga-toxin producing *E.coli (STEC/VTEC) (49)*.

2.4.2. The total aerobic plate count (TAPC)

The total aerobic plate count (TAPC) has been used to indicate general sanitation, effectiveness of intervention steps, microbiological quality, and spoilage in cooked RTE foods, pasteurized milk, and spices. This indicator has also been referred as mesophilic aerobic bacteria (MAB) or standard plate count *(12, 129)*.

Based on food type and the processing/handling of the food, Health Canada categorizes TAPC in to three. Category 1 foods are RTE and are comprised entirely of components that have been cooked in the preparation of the final product without subsequent handling or processing of any kind prior to distribution or sale (ex. soups, bread, quiche, cooked meat, fish & seafood and vegetables*(49)*. The limit of TAPC in RTE foods that lie under category 1 is 10^4 CFU/g, $<10^5$ CFU/g, $\geq 10^5$ CFU/g and classified as satisfactory, marginal and, unsatisfactory respectively. For food that fall under category 2 the microbial guidelines are 10^6 CFU/g, $<10^7$ CFU/g and $\geq 10^7$ CFU/g for satisfactory, marginal and unsatisfactory respectively. Category 2 foods contain some components that have been cooked, but may have been further handled prior to or during the preparation of the final product. This category also applies to any foods that are assembled from RTE foods (excluding those in category 3) that are not subsequently cooked (ex. hot dogs, sandwiches, burgers). Since foods in category 3 contains naturally high numbers of bacteria where it is expected that high standard (aerobic) colony counts would be. Therefore, categorization of TAPC is not applicable. Foods in this group includes fresh fruits or vegetables, deli meats, fermented foods, chicken salad all kind of sprouts and cultured dairy products or any food product incorporating these foods (such as sandwiches *(49)*.

2.5. Sampling and Detection of Listeria in the RTE Food Processing Environment

2.5.1. Sampling Scheme

The design of the environmental testing program and response to a positive finding are among the principal factors that determine effectiveness of *listeria* control program. A variety of schemes for sampling the environment are used throughout the food industry *(134)*.

According to the CFIA, ahead of an environmental inspection, at least 24 hours' notice is required to ensure that a packaging line is in operation and that the establishment can schedule for breaks during production for swabbing. The surfaces must be swabbed three hours or more after the start of the operation. This will provide a reliable assessment of the working conditions as the elapsed time will have allowed surfaces to be inoculated *(16)*. Depending on the volume of production, if the time of production is to be completed within three hours, sampling must be taken in the second half of the production shift. All kits used for sampling must be used before the expiration dates. The sampling sites should include areas that have been found to be good indicators of control *(16)*. The commonly used sampling sites to verify sanitation in the environment are Food Contact Surfaces (FCS) and Non- Food Contact Surfaces (NFCS) are. FCS are areas in the processing environment that comes in direct contact with exposed RTE product and NFCS or Indirect

Food Contact Surfaces are areas adjacent to a FCS, but does not come in direct contact with the product *(148)*.

Depending on the complexity of processing system or packaging line the number of sampling sites vary between 5 to 10. However, it is recommended a minimum of 5 sites of food-contact surfaces in each production line for RTE foods and 5 sites for non-food contact surfaces where RTE foods are processed, exposed, or stored *(148)*. CFIA recommends a 30 x 30 cm or equivalent surface for swabbing *(16)*. Whereas, FDA recommends 30-100 square centimeters (5-15 square inches) in size for sponging and an area that is 10-13 square centimeters (1.5-2 square inches) for swabbing. Sampling areas should prioritized on the basis of areas, where the risk of post processing contamination is higher *(145)*. Different guidelines suggested sampling sites for food contact surfaces including but not limited to slicers, carts/racks, packaging tables, conveyor belts, cutting tables, employee gloves/hands, aprons and NFCS includes drains, floors, walls, and ceilings *(16, 145, 148)*.

2.5.2. Detection of foodborne pathogens

Pathogen detection methods are required for an effective monitoring of microbial pathogens in food supplies in which commonly used traditional detection methods depend upon selective plating combined with immunological or biochemical identification *(2)*. Even if different techniques are used, usually culturing and isolation of the pure isolate is considered as a reference method used for bacteria *(144)* and there are certain methods used as screening techniques that give a quick result and further use culture technique to confirm the positive tested samples *(139)*.

Despite the variation in the sensitivity and specificity of both tests, PCR-based methods are generally known by their fast, efficient and reliable methods used for detection of *L.*

monocytogenes from samples collected in the food processing environment *(92)*. A study also reported about, the real-time PCR method with 99% specificity, 96% sensitivity and 99 % accuracy when compared to the standard culture method *(93)*. Another study also reported detection of similar number of positive samples using RTi-PCR and the conventional culture method *(4)*.

Given the different sampling sites and laboratory techniques, a study reported that RTi-PCR detect *L. monocytogenes* from FCS and NFCS samples were more accurately than the food and raw material samples from the first enrichment *(29)*. Reports from comparative study of RTi-PCR and culture method for detection of *Salmonella* also revealed that the RTi-PCR was, more accurate compared to the culture method with the detection probabilities of 70% and 100% when a *Salmonella* cell suspension in the PCR (5 CFU per reaction) was 10^3 CFU/ml and $10\ ^4$CFU/ml respectively *(76)*; a sensitivity of 0.04 CFU/g *(23)* and 2–4 cells/25 g *(8)*. The common limitations of the culture method are, they are time consuming, laborious, and take several days without isolate confirmation and difficulty to process large volume of samples *(2, 8)*.

2.6. Risk Factors Affecting Microbial Contamination of Foods

Good knowledge of the microbial contamination during any of the steps in the farm-to-table continuum including production, processing, wholesale storage, transportation or retailing and handling in the home as well as retrospective case studies are important to obtain a better understanding of problems and issues that can lead to food-borne illnesses *(94, 108)*. Occurrence of food borne related diseases could be attributed to activities at farm level i.e. initial production step due to pre-harvest sources or from post-harvest contamination *(3)*. Thus, knowledge is essential to design safe food products and manufacturing processes, to correct errors occurring in production and to, when necessary, improve the implemented preventive measures *(7, 108)*.

The FDA indicated the importance of the five broad categories of factors that affect the food safety, known as "foodborne illness risk factors". These include food from unsafe sources, inadequate cooking, improper holding temperatures, contaminated equipment and poor personal hygiene *(146)*. These factors largely affect the food safety within retail and food service establishments. Based on factors that contributed to foodborne illness in Canada, Health Canada has developed the first edition of the Risk Categorization Model and identified eight categories of risk factors. These factors are linked to types of food and intended uses, food preparation and processing, equipment and facility, management and employee food safety knowledge, food safety, management program, regulatory compliance, volume of food and typical patronage *(48)*. Categorizing as the non-human and human risk factors associated with foodborne illness, Lukacsovics et al. (2014) reported poor personal hygiene, cross-contamination, Improper time / temperature control and unsafe food sources as potential risk factors contributing to foodborne illnesses in Canada's food system *(3)*.

CFIA's Science Branch technical committee prepared a risk assessment model to assess the food safety risk of food producing establishments. The model does include detailed criteria, selected and weighted based on a systematic science-based process, that reflected a balanced combination of inherent (initial) risk associated with the products and processes, as well as the track records of the establishment and the performance of their quality system to mitigate the initial risk *(109)*.

The commonest risk factors mentioned in different literatures are types of food and intended use, food form unsafe source, improper holding temperature, equipment and facility and poor personal hygiene *(24, 49, 109, 141)*.

2.6.1. Type of Food and Intended Use

Some foods are more likely to be contaminated with pathogenic microorganisms and to support their growth *(128)*. The types of food being handled in a food establishment is thus important in identifying the hazards likely to be associated with that establishment *(143)*. High or medium risk foods that do not receive further heat treatment will likely be sources of foodborne illness compared to those food items which undergo additional steps to reduce or control microbial growth. Risky food items that receive further heat treatment or undergo other methods to reduce microbial pathogens are also at reduced risk. Low risk foods are usually are not less likely to be involved in a foodborne illness *(48, 143)*. Whether the food is intended to be RTE or not, it`s important in determining the severity of the risk. Ready-to-eat food can present a greater risk of causing foodborne illness as it is not intended for further heat processing *(128)*.

2.6.2. Food from Unsafe Sources

The term "foods from unsafe sources" usually refers to RTE foods that are produced or processed from restaurants, supermarkets, food establishments in a way that does not kill pathogens or that are cooked and waited for some time till they are served. Different studies indicated an association between foods obtained from unsafe sources and occurrence of foodborne illnesses and outbreaks *(11, 32)*.

Food could be mishandled in several ways along the food production chain. Following contamination of foods, undercooking the food, leaving the food at room temperature and other mishandling of the food could enhance the occurrence of outbreaks. Many pathogens grow quickly in food held at room temperature; a few number can grow to a large number in just a few hours. Reheating or boiling food after it has been left at room temperature for a long time does not always make it safe because some pathogens produce toxins that are not destroyed by heat *(138)*. The risk of foodborne illness can be mitigated by following strict food safety behaviors by all stakeholders, including food processors, food retailers and food service personnel *(79)*.

2.6.3. Improper Holding Temperatures

When foods are cooked, most competing microorganisms and pathogenic microorganisms are killed. However, the heat due to improper holding temperature of foods and or development of resistance of the spores allows certain microbes to survive and grow in a noncompetitive environment. Foods like cooked cereals, meats, gravies, and cooked dairy products that are kept at critical temperature range that favors the growth of microbes should be as low as possible so that it limits the growth of pathogens. This

optimal growth temperature range is from 21°C (70°F) to 49°C (120°F). In this temperature range, doubling time for these organisms is only 7 to 10 minutes and growth leads quickly to the numbers sufficient to induce symptoms of gastroenteritis The cooking temperature of raw animal foods such as eggs, fish, meat and poultry with the aim of heating all parts of the food and destruction of pathogenic microorganisms can be achieved at 63°C (145°F) or above for 15 second and or 68°C (155°F) for 15 seconds *(142)*.

According to the FDA Food Code 2013, various temperature requirements were established to maintain the food safety at different levels. Refrigerated, time/temperature control for safety food shall be at a temperature of 5°C (41°F) or below when received. A specific temperature other than 5°C (41°F) for a time/temperature control for safety food is required when law governing its distribution, such as laws governing milk and molluscan shellfish are available. Raw eggs shall be received in refrigerated equipment that maintains an ambient air temperature of 7°C (45°F) or less. Time/Temperature control for the safety of food requires that it is cooked to a temperature and for a time specified and received hot shall be at a temperature of 57°C (135°F) or above. A food that is labeled frozen and shipped frozen by a food processing plant shall be received frozen. Generally, upon receipt, time/temperature control for safety food shall be free of evidence of previous temperature abuse *(142)*. Taken together, these data and guidelines illustrates how the temperature of either the food processing environment, while food is offered at the retail level or at restaurant and even at home, at the consumer level, is closely related with the increased likelihood of bacterial growth and food spoilage. It explains why many Critical Control Points in the food processing activities are related to cooking or cooling steps *(154)*.

2.6.4. Equipment and Facility

It is known that various equipment and facilities, including utensils and surfaces in food establishments are known factors that may be the source of food contamination and there by foodborne illnesses and outbreaks *(98, 113)*. Equipment must be adequate to the volume of food preparation or processing. Refrigeration must be appropriate to its intended use and capable of maintaining required temperatures. The use of commercial equipment built to international standards and certified by third parties is as well recommended. Using the equipment for longer period decreases the efficiency as compared to the new one. Therefore, periodic checkup is mandatory. A well, processing may become less efficient mainly due to old age equipment and it contributes to an increased risk of foodborne illness *(48)*. A positive detection of *L. monocytogenes* was linked with different samples collected within the food processing establishment including knives, mincer and conveyor belts *(99, 110)*.

2.6.5. Poor Personal Hygiene

Food handlers play significant role in addressing food safety during food production and distribution. Food handlers may be involved in contamination or cross-contamination of different types of foodstuffs including inadequately cooked and stored foods and can also be asymptomatic carriers of food poisoning organisms*(24, 27)*.

Maintaining personal hygiene is an important factor that prevents occurrence of foodborne related illnesses *(50)*. Employee practices such as eating, drinking, and smoking in food preparation areas and working while experiencing persistent coughing and sneezing are among the factors that enhance food contamination. Elimination of these practices will

help prevent the transfer of microorganisms to foods and food-contact surfaces *(141)*. Annually, about $8.2 billion is lost due to 9.3 million cases of infections that arise from pathogens associated with personal hygiene *(3, 82, 83)*. It was also reported that poor personal hygiene as the third most commonly reported food preparation practice contributing to occurrence of foodborne disease and further claimed that contaminated hands may be the most important means by which enteric pathogens are transmitted *(71)*. However, the use of thorough hand washing and use of glove are shown to be effective in reducing bacterial cross-contamination *(111)*.

2.7. Evidence-Based Food Safety Strategy

2.7.1. Risk Analysis in Food Safety

Risk analysis has paramount importance in continuous improvement of food safety through improving the process of food safety policymaking and public health. It provides a framework to effectively assess, manage and communicate food safety risks in cooperation with various stakeholders involved. It helps to establish applicable scientific measures to reduce the incidence of food-borne disease, plan and implement mandatory interventions, and monitor successful or unsuccessful outcomes of these interventions *(39)*.

2.7.2. Risk Assessment of Food Establishments

Risk assessment is a scientifically-based process consisting of hazard identification, hazard characterization, exposure assessment and risk characterization. These steps are important in systematic identification of adverse health effects and associated probabilities arising from consumption of foods contaminated with microbial pathogens and/or microbial toxins *(39, 58, 67)*. It also uses scientific findings to determine the likelihood and magnitude of harm attributed to a specific hazard *(30)*. Most modern Food Safety Control Systems are based on risk analysis at least in part since risk assessment is a key component of any food safety management program.

2.7.3. Food Safety Control System

Effective application of food control system aims to protect the consumer from consumption of unsafe food that does not fulfill the safety and quality requirements, and are not labelled as prescribed by law *(40)*. Although significant efforts are put in place to address food safety issues, a huge burden still exists mainly due to microorganisms.

Microbes get access to the food chain at different steps and remain highly adaptable to the environment. Management of food safety, either at the establishment or at the country level, is based on generally accepted principles of risk management. Hazard Analysis Critical Control Points is the most internationally recognized food safety management system. Some establishments rely on Good Manufacturing Practices *(46)*.

2.7.6. Performance Evaluation of Food Safety Control System

Formulation and implementation of food safety management systems to prevent, eliminate, or reduce the occurrence of foodborne illness risk factors is vital to achieve managerial control. Consistent application of such regulatory inspections and follow-up activities is mandatory *(140)* and its effectiveness and relevance to the national food control system should be regularly assessed against the objective of the system, efficacy of the control programs, as well as against legislative and other regulatory requirements. A set of criteria and standards for assessment should be established, clearly defined and documented, and may also include cost benefits and efficiency *(26)*.

HACCP is believed to be an effective and rational tool used to assure food safety, which can be applied throughout the food chain from primary production to final consumption *(33)*. Despite the implementation of HACCP by various food companies, its effectiveness is not well evaluated by the establishments *(20)* and the changing environment with in food establishments and the high requirements on food safety, forced companies to critically assess and improve the performance of their food safety management system (FSMS) *(97)*.

In addition to the quality assurance standards and guidelines used during designing of the control system, P.A. Luning et al. (2015) recommended an independent performance assessment using a tool that comprises of various indicators that help to analyze different

components of the food safety including preventive measures, intervention processes, and monitoring systems, and to analyze actual implementation of these control strategies, i.e. the core control activities. It also includes indicators to analyze the core assurance activities, i.e. setting system requirements, validation, verification and documentation & record keeping *(96)*. Thus, microbiological testing is a necessary part of HACCP implementation, as testing must be used to investigate the microbiological effects of the operations in or affecting a process, to validate the procedures adopted for controlling microbiological contamination, and to verify the maintenance of control over the microbiological condition of product. Such are the proper and necessary uses of microbiological testing for assuring the safety *(73, 125)*.

2.8. The Food Safety Regulatory System in Canada

The Canadian government use and promote science based risk assessment to protect the occurrence of food borne related threats. The industry also take part being transparent about the safety of their product and provision of appropriate information about the health risks and benefits associated with food so as to allow the consumers get the opportunity to make their choices *(38)*. Government agencies namely, Public Health Agency of Canada (PHAC), Health Canada (HC), and the Canadian Food Inspection Agency (CFIA) are primarily responsible to respond on issues related to food-borne illness *(103)*. Outbreaks related to food and follow up investigation are initially addressed by the Centre for Food-borne, Environmental and Zoonotic Infectious Diseases (CFEZID), within the Infectious Disease Prevention and Control (IDPC) branch of PHAC *(103)*.

Assessment of any kind of potential risk in the food which might have public health significance and mitigation procedures for such a risk is authorized and implemented by CFIA. The CFIA coordinates food recalls with external food safety partners in which about 350 recalls each year managed *(17)*.

The safety and nutritional quality of all food sold in Canada are managed by the rules and regulations developed by HC and the CFIA is mandated to enforce those policies and standards *(37)*. The CFIA designs, develops and manages programs related with inspection and service standards, including supplying laboratory support. It also deals with different government, industry and trading partnerships, with respect to inspection and compliance programs, and provides laboratory support *(37, 38)*.

The Agriculture and Agri Food Canada (AAFC) mainly promotes and provides information, on various research and technology related policies that help to achieve a secure food system, health of the environment and innovation for growth *(38)*.

In the Provincial and Territories (P/Ts) context, foodborne related outbreak reporting is done by the local/regional health officials. These personnel also conduct inspection and different awareness activities to reduce risks related to food and when needed request assistance from HC, PHAC, or the CFIA usually in the response to a potential food-borne illness outbreak. In certain P/Ts, other departments (including Agriculture and Agri-Food) may also have a role in food-borne illness investigations *(103)*. Whenever, there is a need for a centralized data collection on foodborne outbreak, the P/Ts provide the case-level information to the respective body *(103)*.

2.8.1. The Quebec Risk Based Food Inspection

There are about 179 federally registered food processing plants, 31 slaughter houses and 33 food storage sites in Quebec inspected by CFIA *(18)*. The Quebec risk based inspection (RBI) was developed by creating a new mathematical algorithm that was inspired by the French, English and USA food inspection systems. Since its implementation in1996, the RBI method applied to all food establishments under provincial jurisdiction subject to inspection programs *(77)*. Approximately 61,966 active establishments of the sector "Food and Retail" are inspected by the MAPAQ and the city of Montréal. These establishments include but not limited to cafeteria, hospital, butchery, dairy, bakery, fish, vending machine, public market, kiosk, grocery store, supermarket, food truck, snack, sugar shack, daycare, camp holiday, reception, catering, outfitting, chocolate and dairy bar *(77)*. The RBI method respects the fundamentals of the risk-based

inspection and includes different control points and prerequisite programs and may also include inspection of food. It does not however, impose the application of a full HACCP program since many of the small-scale facilities does not possess the resources needed for a full HACCP implementation. The inspection is conducted on the basis of fixed regular interval, regular interval based on the risk level or based on complaint from the public and it assess control points in the establishments *(86)*.

The risk level of a food establishment is calculated from the assessment of risk factors and sub-factors made by the inspector. A value is assigned to these factors and sub-factors based on the type of establishment; the multiplicity and complexity of operations; implanted monitoring and control measures; the number of food handlers; the type of food products; the extent or volume of activities and compliance history. The approach considers the risk to human health posed by food and is based on international reference standards. As the operator is responsible to control risks within the establishment; the inspector establishes his judgment from requirements (law on foodstuffs and regulations or guidelines) which determine the applicable "control measure" considering the lack of controls *(86)*.

2.8.1.1. Components of RBI and Determination of Risk level

The MAPAQ risk-based inspection consists of two main steps, assessment of control points and assessment of the risk level. The assessment of control points is related to the inspection of the control points and to collect, if applicable, the information required to assess various factors and sub-factors that determine the level of risk of the establishment. It also verifies the control of risks related to sources of risk in terms of hygiene and food preparation practices. The RBI method combines these sources of risk to 23 control points These 23 control points are grouped within five categories, also named

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5M: Material, Methods, Manpower, Equipment and Environment (Annex 1). Upon inspection, the criteria associated with each of the control points are checked by the inspector to determine the level of the risk and the score of 10 or 50 will be given and graded as major or grave (serious) respectively *(87)*. The assessment of the risk level (if applicable) step mainly evaluates the factors and sub-factors leading to the calculation of the risk burden for the establishment subjected to regular inspection and rank establishments based on their risk level in to low, medium low, medium, medium high and high risk establishment. The risk level is calculated by the computer system by integrating the values of factors and sub-factors assigned by the inspector or the system.

2.9. Objectives and Hypothesis

2.9.1. General Objective

The objective of this study was to estimate the relationship between the score of control points in the MAPAQ risk assessment model and the presence pathogenic and load of hygiene indicator microbes in the environment within the food establishment.

2.9.2. Specific Objectives

1. To estimate the association between the presence of *Listeria monocytogenes* and selected control point scores of the MAPAQ model

2. To estimate the association between the presence of *Salmonella* and the selected control point scores of the MAPAQ model.

3. To estimate the association between the load of *E. coli* and the selected control point scores of the MAPAQ model.

4. To estimate the association between the load of *total aerobic count* and selected control point scores of the MAPAQ model.

5. To estimate the relationship between the total scores of control points and *Listeria monocytogenes, Salmonella, E. coli,* and *total aerobic count.* One the basis of the above objectives, the hypothesis was formulated as shown below.

H_0: There is no relationship between between the MAPAQ model scores and presence of *Listeria monocytogenes* and *Salmonella* and load of *E. coli* and mesophilic bacteria count as indicator microorganisms.H_1: There is a relationship between the MAPAQ model scores and presence of *Listeria monocytogenes, Salmonella* and load of *E. coli* and total aerobic bacteria count.

CHAPTER 3. MATERIALS, METHODS AND RESULTS

ASSOCIATION BETWEEN PATHOGENIC AND INDICATOR BACTERIA AND SOME CONTROL POINTS OF A RISK ASSESSMENT MODEL FOR FOOD PRODUCING ESTABLISHMENTS IN QUÉBEC, CANADA

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Abstract

Mitigating the risk of foodborne pathogens in food processing establishments is an essential part of food safety control system from an establishment or a food control agency perspective. The objective of this study was to estimate the association between selected microorganisms and scores from 20 control points of a risk assessment inspection model. A cross-sectional study was conducted on a convenient sample of 18 meat processing ready to eat (RTE) establishments food establishments located in Quebec between June and July 2015. Sponge swabs of 900 cm^2 in surface area of three food contact and two non-food contact surfaces were sampled per establishment. *Real Time-PCR* was done using SYBER Green, targeting the *siiA* gene of *Salmonella* and both *prfA* and *prs* genes of *Listeria monocytogenes*. We detected one *Salmonella (1 %)* and 18 *L. monocytogenes (20 %)* isolates from the 90 samples collected in all establishments. *E. coli* was present in 7 of 18 (39%) establishments. The mean Log10 counts of *E. coli* and Total Aerobic Count (TAC) were 1.16 and 6.01, respectively. Among control points assessed by inspectors, "*ambient temperature*" and the presence of "*potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants*" showed a positive relationship with the level of *L. monocytogenes* ($p = 0.03$) and *(p= 0.04)* respectively. *E. coli* was also positively associated with the control point *"ambient temperature" (p =0.02)* and *"environmental sources of contamination"* ($p = 0.005$). The presence of adequate hand washing facilities was linked to the load of TAC ($p = 0.01$) in the establishments. This study provides new insight on the relationship between inspector assessment of specific control points and microbiological load in food establishments.

Introduction

It is estimated that annually four million (1 in 8) Canadians get sick due to domestically acquired foodborne infections *(41)*. Of these, some data suggest as much as 11, 600 annual hospitalizations and 238 deaths occur *(35, 41)*. The Hazard Analysis and Critical Control Point (HACCP) and/or related hygienic programs (good hygienic practices, GHP) are the major tools used in different small or mid-scale food establishments, such as those inspected by provincial authorities in Canada, so as to prevent undesired food safety outcomes *(16)*. The main target of these systems is to identify and control consumer safety hazards in the production line and within the establishment environment to ultimately ensure a safer product for consumption *(16, 20)*. The performance of an establishment food safety control system (FSCS) is usually assessed by different preset requirements via audits/inspections and also, in some instances by microbiological analysis *(34)*. Indeed, inspection of the FSCS can be performed without conducting systematic microbiological analysis *(21)*. However, microbiological analysis, as an adjunct for the assessment of the performance of these systems, has proven to be an invaluable approach in strengthening the audit/inspection results *(18, 20, 34)*.

Salmonella and *Listeria monocytogenes* account for 17% and 35% of foodborne related deaths in Canada, respectively *(40)*. The likelihood of the presence of *Salmonella* in a food establishment and the overall hygiene status of an establishment is often addressed through indirect analysis of *E. coli* and Total Aerobe Count (TAC), in which these microbes are known as hygiene indicators *(9, 14)*. Whenever there is a significant load of bacteria on the food contact surfaces, the probability of cross-contamination to food increases *(24, 27)*. Studies on distribution of fecal indicators such as *E. coli* and food-borne pathogens in the production

facilities and environment may provide valuable insights for policy makers within regulatory agencies working on food safety related issues *(3, 12)*.

The Ministry of Agriculture, Fisheries and Food (Ministère de l'Agriculture, des Pecheries et de l'Alimentation du Quebec- MAPAQ) of the Quebec province has been implementing a comprehensive risk based inspection system 1996 for food producing establishments located in this province. The model ultimately categorizes establishments based on their risk profile as high, medium and low risk levels *(33)*. The objectives of this study were to assess the relationship between selected inspection factor control points of the MAPAQ risk assessment model and microbiological contamination (extent of *Salmonella* and *L. monocytogenes*, load of indicator *E. coli* and *total aerobic count*) in the environment within food establishments.

Materials and Methods

Sampling plan. A cross-sectional study was conducted between June, 2015 and July, 2015 in RTE food establishments located in Laval and south shore of Montreal in Quebec, Canada. Conveniently, along with MAPAQ regular and follow up inspection activities, 18 food establishments that process and prepare meat and ready to eat (RTE) foods including patty, deli meat, sausage, meat based pasta sauce, marinated poultry were selected. Five sponge swab samples including 3 food contact surfaces (FCS) and 2 non-food contact surfaces (NFCS) per establishment were collected for a total of 90 samples. Food contact surface samples were taken from processing table, conveyor and slicer / knife, whereas NFCS included floor of the processing room and of the refrigeration room as previously reported *(7)*. According to the Canadian Food Inspection Agency (CFIA) sampling guideline*s (4)*, 30 cm x

30 cm area of these surfaces were swabbed using the pre-moistened sterile sponge-stick (3M. St. Paul, Minnesota, USA). All samples were collected aseptically wearing gloves and appropriate clothing. Each sample was packed separately, placed in a cool box with ice packs and, within 4 hours of collection, transported to the laboratory of the research chair in meat safety (CRSV) of the Université de Montréal.

Listeria monocytogenes **detection.** *L. monocytogenes* detection was done by conducting a parallel culture and RTi- PCR approach. A two-steps enrichment procedure, as recommended by the Health Canada MFHPB-30 standard technique was performed. As described by Lariviere-Gauthier *et al. (28)*, University of Vermont medium 1 (UVM-1; Lab M, Heywood, United Kingdom) was used as the first enrichment in which sponge swabs were initially put and incubated at 30°C for 48 hours. From the incubated broth, 0.1ml of primary enrichment broth was inoculated in to the second enrichment, Fraser broth (Lab M, United Kingdom) and again incubated for 24 h at 37°C. Loop fulls of enriched broths were spread on ALOA agar plates (ALOA; AES Chemunex, Bruz, France) and incubated for 48 h at 37°C. Following incubation, ALOA agar plates were thoroughly examined for typical *L. monocytogenes* colonies (blue-green colonies with halo). Presence of *L. monocytogenes* DNA was also confirmed by RTi-PCR. Multiplex *RTi-PCR* analysis, targeting the *prfA* and *prs* genes *(25, 28)* was conducted to identify and confirm *L. monocytogenes* from 2ml of the secondary enrichment broth of all samples.

Salmonella **detection.** Sponge swabs were placed in sterile bags containing buffered peptone water and incubated for 24 h at 37 °C. Detection of *Salmonella* DNA was performed,

as described in Table 1.   Using 2ml of the secondary enrichment broths, all samples were

analyzed by *RTi-PCR* using the *siiA* gene as described by Hessena et al. *(1)*.

For *Salmonella* culture, as described by Letellier et al. and Larivière-Gauthier et al.

(15, 30), samples were put in Rappaport-Vassiliadis (Difco, Detroit, MI) and tetrathionate

brilliant broth (BBL, Becton Dickinson, Cockeysville, MD) selective enrichment broths and

then inoculated on brilliant green sulfa agar (Difco), a selective agar media supplemented with

20 mg/ml novobiocin (Sigma, St. Louis, MO).   Lactose negative colonies were tested for

urease production (Difco) and for typical reaction on triple sugar iron media (Difco).   Colonies

with typical biochemical patterns of *Salmonella* were tested using slide agglutination with a

polyvalent O-antiserum (Poly A1-Vi, Difco).

Enumeration of *E. coli* and Total Aerobic Count *(TAC)*.   The *E. coli* and TAC load was

determined using 3M Petrifilms at different dilutions (3M. St. Paul, Minnesota, USA) based

on the manufacturer's guidelines.   Briefly, 1ml of pre-enriched sample was added and

vortexed in to 9ml of Tryptone Saline Solution.   Dilutions from -1to -6 were prepared and 1ml

of each dilution were added on the Petrifilm and incubated at 37 °C for 48 hours.   The

appearance of blue colony with gas was interpreted as confirmed *E. coli and* all red dots

regardless of size or intensity was counted as total aerobic counts *(44)*.

Inspector scores of control points.   Based on the MAPAQ risk based food inspection

model, twenty-three control points under five categories were thoroughly inspected and

assessed by the MAPAQ inspectors *(33)* and sampling was conducted in parallel. Based on the

preset MAPAQ guidelines, each control points were scored with either serious problem, or

with major problem.   When a model control point was left unscored, it was assumed as

fulfilling the minimum hygienic criteria and considered as an acceptable score for our analysis.

Statistical analysis. The RTE food-producing establishment was considered as unit of analysis. Considering their biological relationship with microbial contamination, twenty-one control points were selected for statistical analyses. The associations between inspector score and microbial presence/load were estimated for these control points. Based on both culture and RTi-PCR results from each establishment, the level of *L. monocytogenes* and *Salmonella* were categorized based on the number of positive sampled sites (0, 1-2, $\geq$3) for each establishment. The exact Mantel–Haenszel (MH) or exact Pearson Chi-square was used to estimate the association between the level of *L. monocytogenes* or presence of *E. coli* with the score of control points. Since *E. coli* was found in few establishments, we were forced to use a binomial approach [present (1) and absent (0)] category at the establishment level instead of the load and tested with the scores of control points. The mean load of log10 TAC among all samples was calculated for each establishment. The unequal variances t-test was used in estimating the association between control points and presence and load of microbes. Based on the inspector score of control points in each establishment, we categorized establishments in two categories, those who have a major and serious score for over 4 control points and below 4 control points among the 21 control points. These two categories (<4 and $\geq$4) were tested for a possible presence of an association with the level, presence and load of *L. monocytogenes*, *E. coli* and TAC respectively.

Results

Detection of *L. monocytogenes.*

Based on culture and RTi-PCR analysis, the number of *L. monocytogenes* positive samples from food establishments were 15 (17 %) and 18 (20 %), respectively, from 90 samples. All *L. monocytogenes* culture positive samples were also positive during the *RTi-PCR* analysis. The *RTi-PCR* detected positive samples collected from 11 FCS and 7 NFCS. The type of samples found most often positive for *L. monocytogenes* were both conveyors and processing table (for each n=6) from FCS and processing room floors (n=5) from NFCS. In all establishments, when conveyors were positive, the floor of the processing room was positive too. At the establishment level, 9/18 (50%) tested positive for *L. monocytogenes* using culture and RTi-PCR.

Detection of *Salmonella*. Based on culture, we found only two *Salmonella* positive samples out of 90 samples (2 %) and these samples were taken from one chicken processing table and floor of the processing room. However, using *RTi-PCR,* we confirmed only that sample from chicken processing tableout of 90 samples (1%). *Salmonella* was detected only in one of the establishments out of 18 (6%).

Load of *E. coli* and TAC. The mean Log10 counts of *E. coli and* TAC were 1.16 and 5.15 respectively from all establishments.

Association between inspector scores of control points and microorganisms. Among the various control points selected, "ambient temperature", "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants during

preparation", "environmental sources of contamination" and "presence of adequate hand washing facilities" were found associated with at least one of the various microorganisms studied.

Both the control points "ambient temperature" and "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants during preparation" with major and serious problem showed a significant positive association with a higher probability of recovering *L. monocytogenes* ($p = 0.03$) and ($p = 0.05$) respectively. The presence of *E. coli* was also positively associated with "ambient temperature" ($p = 0.02$) and "environmental sources of contamination (chemical, physical or microbiological)" ($p = 0.005$). The t- test also indicated a positive association between the load of *TAC* ($p = 0.01$) and the control point "presence of adequate hand washing facilities" in the surveyed establishments. Since we found *Salmonella* in only one establishment, we were unable to perform and compare with the inspection score and control points.

The total number of control points with a major and or serious score in each establishment was also tested to estimate its association with presence and or load microbes. However, no statistically significant association was observed ($p =1.0$; $p= 1.0$ and $p= 0.3$ were for *L. monocytogenes*, *E. coli*, and TAC respectively).

Discussion

In this study, *L. monocytogenes* was isolated in roughly half of sampled establishments accounting 12.2 % of the FCS and 7.8% of NFCS samples (n=90). Even though the current study did not aim to estimate the prevalence of this microorganism, there were other studies in Canada, conducted in British Columbia, that showed a prevalence of *L. monocytogenes* on

FCS as 25% (3/12) and NFCS 83.3% (10/12) in establishments. In Quebec, in the context of a farm to table approach, the prevalence of *L. monocytogenes* in FCS and NFCS, specifically within the abattoir environment (lairage pens) was *1.7% (n=600)*. In the same establishments, within the precutting room (samples from the equipment and environmental surfaces) it was 39.4 % (n=33) while in the cutting rooms (equipment and conveyor samples) a prevalence of 71.8 % (n=39) was observed *(26, 28)*. Outside Canada, in a comprehensive study that included six European countries, *L. monocytogenes* was detected in 20% and 37% of FCS (n=177) and NFCS (n=334) respectively. In Ireland, 4.4% (n=1574) of positive *L. monocytogenes* samples were detected from FCS and *(7, 29)*. These findings along with our own results underline the importance of assessing both the FCS and NFCS to assess presence of this foodborne pathogens in food establishment when it is expected to estimate an association between different control points in the food establishment and *L. monocytogenes*.

Although it is not uncommon to find this psychrophilic bacterium in the cold environment of food processing establishments, its presence likely increases the probability of finding it on food contact surfaces and within the product *(5)*. However, in the present study, we have regularly found sites like conveyors and processing tables positive for *L. monocytogenes*. This might be due to cross-contamination with the floor of the processing room or refrigerating room. Since the pathogens that can persist in the harsh conditions of the food processing and refrigeration environment are ubiquitous, the risk of contaminating equipment and cross-contamination to food is expected to be higher *(8, 23)* than normal. Other studies indicated that specific sites like conveyors have been associated with a possible contamination of product contact surfaces during production in commercial RTE meat and poultry producing facilities *(42)*

Given the frequent finding of this bacterium within processing plant environments, especially in those that produce high risk food like RTE products, it is suggested to consider microbiological sampling as an adjunct of regular inspection activities *(43)*. This was supported by our finding in which *L. monocytogenes* were found in some of the establishments that were at low level of risk during inspection-based scores. It worthwhile to mention however that inspection scores such as the one used in this study are designed to also assess the establishment performance to control various types of many microbiological hazards such as STEC and *Clostridium perfringens*. While we have tried to assess the link between the inspection factors that are more likely to be linked to microbial hazards from a biological relevance point of view, it was not unexpected that some risk factors where not correlated with the presence of a psychrophilic bacterium such as *L. monocytogenes*. In addition, given the relatively low number of establishments sampled in this study, it is very likely that an increased number of establishments would have allow us to find more positive associations.

Nevertheless, considering the importance of *L. monocytogenes* as a microbial hazard, and its involvement in recent food outbreaks, some national food control authorities, such as the Canadian Food Inspection Agency, had put in place systematic sampling schemes to detect *L monocytogenes* within the production environment and the product. However, the difficulties related to logistics and cost of applying such systematic sampling schemes in multiple small facilities, such as those under MAPAQ jurisdiction, underline the need to put the emphasize on identifying inspection control points that the more likely to be related with the presence of this bacterium.

The "ambient temperature" control point was associated with a higher probability of detecting *L. monocytogenes*. Improper holding temperatures have been pointed out regularly as a risk factor for the presence of this bacterium in RTE products *(13, 31, 32)*. This finding suggests that compliance to this control point could be used to target at risk establishments for the presence of *L. monocytogenes*. The higher level of *L. monocytogenes* in our study was associated with the control point "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants". This control point comprises of multiple factors that enhance direct or cross contamination of the product including different allergen in the food during preparation. Un-cleaned utensils, use of common processing table for different food products and hand are important causes of cross contamination. The use of similar utensils and cooking oil for different batches of food is also another important factor that results in food contamination and intoxication *(33)*. Since *L. monocytogenes* is a common pathogen found in the environment, there is much higher chance of cross-contamination between environment, equipment and employee hands within food establishments. This could result in the occurrence of cross contamination. Other studies also indicated a positive association between *L. monocytogenes* and different factors linked to cross contamination, whenever the processing environment and the final products was tested positive for the pathogen *(6, 13)*.

In our study, "presence of hand washing facilities", including hand sanitizers were associated with a higher total aerobic count (TAC) in food establishments. Other studies

reported high TAC count from FCS and NFCS and association with noncompliance with hand washing facilities within the food establishment *(37, 38).*

The control point "hand washing and behavior of people in food establishment" was also tested. However, neither listeria nor indicators show statistically significant association. It is possible that a higher number of establishment would have made possible to find a positive association but it is conceivable that even if employees have good awareness about the importance of personal hygiene, shortage of facilities used for sanitations inside the establishments potentially compromise the overall personal hygiene principles within the food establishment.

Identification of *Enterobacteriaceae* in the food processing environment, an area known as an important source of recontamination, provides vital information regarding pathogen identification and preparation of early warning procedures related to food safety *(36)*. We also found an association between the control point "environmental sources of contamination" and *E. coli* presence in food establishments. Another study also reported the *E. coli* presence in the retail and food processing plants environment *(11)*. Other studies also reported such an association between indicator aerobic and pathogenic bacteria with different FCS and NFC in food establishments *(2, 10).* In addition, aerobic bacteria are usually abundant among microbiomes from different compartments of the production plant environment *(19)*. Abrasions on different types of material used in the establishment lead to surface roughness that makes cleaning challenging and could serve as a suitable niche for microbes because of increased numbers of attachment sites *(17)*; it may contributes to contaminate the product or other surfaces within the establishment.

The total number of control points with a major and or serious score in each establishment was not associated with any of the microbial analysis. It is always very difficult to interpret negative results in a survey with a limited number of establishments. On one side, as indicated earlier, the inspection model used in this study is designed to assess control of many microbiological hazards as well as chemical and physical ones. Nevertheless, on the other side the concept of microbial testing as part of inspection based assessment of control points was addressed by other studies questioning the degree of value of inspection by finding a lack of association between visual inspection ratings of different control points and the and microbial results from food establishments *(22, 39)*. These findings and our own results, suggest the need of a microbial sampling scheme as a complement of risk ranking of food establishments, particularly when a microorganism such as *L. monocytogenes* is concerned.

In conclusion, a positive association was found between *L. monocytogenes*, *E. coli* and TAC and some of the MAPAQ model control points, namely "ambient temperature", "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants, "environmental sources of contamination" and "presence of adequate hand washing facility" and in RTE food establishments. Therefore, it is suggested that food regulation agencies consider results of our microbial analysis in targeting risk factors and/or control points that may be linked to the presence of *L. monocytogenes* in small scale food establishments that process and prepare meat RTE food products.

References

1. Ben Hassena A., M. Barkallah, I. Fendri, N. Grosset, I. Ben Neila, M. Gautier, and R. Gdoura. 2015. Real time PCR gene profiling and detection of Salmonella using a novel target: The siiA gene. *J. Microbiol. Methods*. 109:9-15.

2. Bobe G., D. J. Thede, T. A. Ten Eyck, and L. D. Bourquini. 2007. Microbial levels in Michigan apple cider and their association with manufacturing practices. *J. Food Prot.* 70:1187-93.

3. Brown M. H., C. O. Gill, J. Hollingsworth, R. Nickelson, S. Seward, J. J. Sheridan, T. Stevenson, J. L. Sumner, D. M. Theno, W. R. Usborne, and D. Zink. 2000. The role of microbiological testing in systems for assuring the safety of beef. *Int. J. Food Microbiol.* 62:7-16.

4. Canadian Food Inspection Agency. 2013. Environmental and Finished Dairy Product Sampling Monitoring for *Listeria monocytogenes*. Available at: http://www.inspection.gc.ca/food/dairy-products/manuals-inspection-procedures/product-inspection/monitoring-for-listeria-monocytogenes/eng/1335465632611/1335466303763?chap=0. Accessed 3 February, 2016.

5. Chasseignaux E., P. Gerault, M. T. Toquin, G. Salvat, P. Colin, and G. Ermel. 2002. Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. *FEMS microbiology letters*. 210:271-5.

6. Chen B. Y., C. Y. Wang, C. L. Wang, Y. C. Fan, I. T. Weng, and C. H. Chou. 2016. Prevalence and Persistence of *Listeria monocytogenes* in Ready-to-Eat Tilapia Sashimi Processing Plants. *J. Food Prot.* 79:1898-1903.

7. Dalmasso M., A. S. Bolocan, M. Hernandez, A. E. Kapetanakou, T. Kuchta, S. G. Manios, B. Melero, J. Minarovicova, M. Muhterem, A. I. Nicolau, J. Rovira, P. N. Skandamis, B. Stessl, M. Wagner, K. Jordan, and D. Rodriguez-Lazaro. 2014. Comparison of polymerase chain reaction methods and plating for analysis of enriched cultures of *Listeria monocytogenes* when using the ISO11290-1 method. *J. Microbiol Methods*. 98:8-14.

8. Dara Leong Avelino Alvarez-Ordóñez, Piet Jooste and Kieran Jordan. 2016. *Listeria monocytogenes* in food: Control by monitoring the food processing environment. *Afr. J. Microbiol Res.* 10:1-14.

9. Delhalle L., L. De Sadeleer, K. Bollaerts, F. Farnir, C. Saegerman, N. Korsak, J. Dewulf, L. De Zutter, and G. Daube. 2008. Risk factors for *Salmonella* and hygiene indicators in the 10 largest Belgian pig slaughterhouses. *J. Food Prot.* 71:1320-9.

10. Domenech-Sanchez A., E. Laso, M. J. Perez, and C. I. Berrocal. 2011. Microbiological levels of randomly selected food contact surfaces in hotels located in Spain during 2007-2009. *Foodborne Pathog. Dis.* 8:1025-9.

11. Fluckey W. M., M. X. Sanchez, S. R. McKee, D. Smith, E. Pendleton, and M. M. Brashears. 2003. Establishment of a microbiological profile for an air-chilling poultry operation in the United States. *J. Food Prot.* 66:272-9.

12. Fu L. L., and J. R. Li. 2014. Microbial source tracking: a tool for identifying sources of microbial contamination in the food chain. *Crit. Rev Food Sci Nutr*. 54:699-707.

13. Gallagher D., R. Pouillot, K. Hoelzer, J. Tang, S. B. Dennis, and J. R. Kause. 2016. *Listeria monocytogenes* in Retail Delicatessens: An Interagency Risk Assessment-Risk Mitigations. *J. Food Prot.* 79:1076-88.

14. Ghafir Y., B. China, K. Dierick, L. De Zutter, and G. Daube. 2008. Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *J. Food Prot.* 71:35-45.

15. Guillaume Larivière-Gauthier Ann Letellier, Étienne Yergeau, Benoît Laplante and Philippe Fravalo. 2015. Evolution of *Salmonella* excretion by sows during gestation in link with the faecal microbiome. *In*, Safepork 2015 Conference Lisbon, Portugal.

16. Herrera A. G. 2004. The hazard analysis and critical control point system in food safety. *Methods Mol. Biol.* 268:235-80.

17. Holah J. T., and R. H. Thorpe. 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. *J. Appl Bacteriol.* 69:599-608.

18. Holvoet K., L. Jacxsens, I. Sampers, and M. Uyttendaele. 2012. Insight into the prevalence and distribution of microbial contamination to evaluate water management in the fresh produce processing industry. *J. Food Prot.* 75:671-81.

19. Hultman J., R. Rahkila, J. Ali, J. Rousu, and K. J. Bjorkroth. 2015. Meat Processing Plant Microbiome and Contamination Patterns of Cold-Tolerant Bacteria Causing Food Safety and Spoilage Risks in the Manufacture of Vacuum-Packaged Cooked Sausages. *Appl. Environ. Microbiol.* 81:7088-97.

20. Jacxsens L., J. Kussaga, P. A. Luning, M. Van der Spiegel, F. Devlieghere, and M. Uyttendaele. 2009. A Microbial Assessment Scheme to measure microbial performance of Food Safety Management Systems. *Int. J. Food Microbiol.* 134:113-25.

21. Jacxsens L., M. Uyttendaele, F. Devlieghere, J. Rovira, S. O. Gomez, and P. A. Luning. 2010. Food safety performance indicators to benchmark food safety output of food safety management systems. *Int. J. Food Microbiol.* 141 Suppl 1:S180-7.

22. Jones T. F., B. I. Pavlin, B. J. LaFleur, L. A. Ingram, and W. Schaffner. 2004. Restaurant inspection scores and foodborne disease. *Emerg. Infect Dis.* 10:688-92.

23. Jordan M. Dalmasso and K. 2013. Process environment sampling can help to reduce the occurrence of "*Listeria monocytogenes*" in food processing facilities. *Irish J. Agri Food Res.* 52: 93-100.

24. Keeratipibul S., and S. Lekroengsin. 2009. Risk analysis of *Listeria spp.* contamination in two types of ready-to-eat chicken meat products. *J. Food Prot.* 72:67-74.

25. Kérouanton Annaëlle, Muriel Marault, Laetitia Petit, Joël Grout, Trinh Tam Dao, and Anne Brisabois. 2010. Evaluation of a multiplex PCR assay as an alternative method for *Listeria monocytogenes* serotyping. *J. Microbiol. Methods*:134–137.

26. Kovacevic J., L. F. McIntyre, S. B. Henderson, and T. Kosatsky. 2012. Occurrence and distribution of *listeria* species in facilities producing ready-to-eat foods in British Columbia, Canada. *J. Food Prot.* 75:216-24.

27. Kusumaningrum H. D., G. Riboldi, W. C. Hazeleger, and R. R. Beumer. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *Int. J. Food Microbiol.* 85:227-36.

28. Lariviere-Gauthier G., A. Letellier, A. Kerouanton, S. Bekal, S. Quessy, S. Fournaise, and P. Fravalo. 2014. Analysis of *Listeria monocytogenes* strain distribution in a pork slaughter and cutting plant in the province of Quebec. *J. Food Prot.* 77:2121-8.

29. Leong D., A. Alvarez-Ordonez, and K. Jordan. 2014. Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland. *Front. Microbiol.* 5:436.

30. Letellier A., G. Beauchamp, E. Guevremont, S. D'Allaire, D. Hurnik, and S. Quessy. 2009. Risk factors at slaughter associated with presence of *Salmonella* on hog carcasses in Canada. *J. Food Prot.* 72:2326-31.

31. Lianou A., and J. N. Sofos. 2007. A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *J. Food Prot.* 70:2172-98.

32. Menendez R. A., E. Rendueles, J. J. Sanz, R. Capita, and C. Garcia-Fernandez. 2015. Behavior of *Listeria monocytogenes* in Sliced Ready-to-Eat Meat Products Packaged under Vacuum or Modified Atmosphere Conditions. *J. Food Prot.* 78:1891-5.

33. Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec (MAPAQ). 2015. Bonnes pratiques d'hygiène et de salubrité alimentaires Étiquetage. Available at: http://www.mapaq.gouv.qc.ca/fr/Restauration/Qualitedesaliments/securitealiments/inspection/methodeinspection/Pages/bonnespratiqueshygienesalubrite.aspx. Accessed 10 March, 2017.

34. P.A. Luninga L. J., J. Rovirac, S.M. Osésc, M. Uyttendaeleb, W.J. Marcelisd. 2011. A concurrent diagnosis of microbiological food safety output and food safety management system performance: Cases from meat processing industries. *Food Control.* 22:555-565.

35. Public Health Agency of Canada. 2014. Estimating the burden of food-borne illness in Canada. Available at: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/14vol40/dr-rm40-14/dr-rm40-14-comm-eng.php#footnote1. Accessed 15 August, 2016.

36. Reij M. W., and E. D. Den Aantrekker. 2004. Recontamination as a source of pathogens in processed foods. *Int. J. Food Microbiol.* 91:1-11.

37. Rodriguez M., A. Valero, G. D. Posada-Izquierdo, E. Carrasco, and G. Zurera. 2011. Evaluation of food handler practices and microbiological status of ready-to-eat foods in long-term care facilities in the Andalusia region of Spain. *J. Food Prot.* 74:1504-12.

38. Sneed J., C. Strohbehn, S. A. Gilmore, and A. Mendonca. 2004. Microbiological evaluation of foodservice contact surfaces in Iowa assisted-living facilities. *J. Am Diet Assoc.* 104:1722-4.

39. Tebbutt G. M. 1988. Laboratory evaluation of disposable and reusable disinfectant cloths for cleaning food contact surfaces. *Epidemiol. Infect.* 101:367-75.

40.   Thomas M. K., R. Murray, L. Flockhart, K. Pintar, A. Fazil, A. Nesbitt, B. Marshall, J. Tataryn, and F. Pollari. 2015. Estimates of foodborne illness-related hospitalizations and deaths in Canada for 30 specified pathogens and unspecified agents. *Foodborne Pathog. Dis.* 12:820-7.

41.   Thomas M. K., R. Murray, L. Flockhart, K. Pintar, F. Pollari, A. Fazil, A. Nesbitt, and B. Marshall. 2013. Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. *Foodborne Pathog. Dis.* 10:639-648.

42.   Tompkin R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J.Food Prot.* 65:709-25.

43.   U.S. Food and Drug Administration. 2014. Food CGMP Modernization — A Focus on Food Safety. Available at: https://www.fda.gov/Food/GuidanceRegulation/CGMP/ucm207458.htm. Accessed 17 December, 2016.

44.   U.S. Food and Drug Administration. 2015. Bacteriological Analytical Manual. Aerobic Plate Count. Available at: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm. Accessed 5 August, 2016.

Table 1: Summary of methods used to detect Salmonella and L. monocytogenes DNA using RTi-PCR

Pathogens	*Salmonella*	*Listeria monocytogenes*
Volume of enrichment	2ml	2ml
DNA extraction method	Powersoil DNA isolation kit	Power soil isolation kit
DNA analysis method	Hassen *et al.,* 2014	Larivière-Gauthier G. et al.,2014
Primers used	*SiiA*	*PrfA, Prs*
Total reaction volume	20µl	20µl
Volume of DNA extract	5µl	5µl

Table 2: Sources of samples and percentage of positive samples for L. monocytogenes samples from FCS AND NFC surfaces in meat related RTE establishments

Establishment Id	No. of samples	*L. monocytogenes* positive samples	
		No. (%) of positive samples	Sites with positive samples detected
1	5	5 (100)	Processing table, slicer, bone cutting machine, *floor of the processing and refrigeration rooms*
2	5	2 (40)	Conveyor, *floor of the processing room*
3	5	2 (40)	Conveyor, processing table
4	5	-	-
5	5	1 (20)	Processing table
6	5	1 (20)	*Floor of the processing room*
7	5	0 (0)	-
8	5	0 (0)	-
9	5	2 (40)	Knife, *floor of the processing room*
10	5	0 (0)	
11	5	1 (20)	Conveyor
12	5	1 (20)	*Floor of the refrigeration room*
13	5	0 (0)	
14	5	0 (0)	-
15	5	0 (0)	-
16	5	0 (0)	-
17	5	0 (0)	-
18	5	3 (60)	Processing table, conveyor, *floor of the processing room*

Table 3:Controls points scores from 18 establishments and relationship with L. monocytogenes

Inspector evaluation of control points		No (%) establishment per *L. monocytogenes* status			
		0	**1-2**	**3-5**	**p-value**
Ambient Temperature					
Acceptable	15	9 (60)	5 (33)	1 (7)	0.02
Major problem	3	0 (0)	1 (33)	2 (67)	
Internal temperature of the food					
Acceptable	16	9 (56)	5 (31)	2 (13)	0.1
Serious problem	2	0 (0)	1 (50)	1 (50)	
Safety					
Acceptable	18	9 (50)	6 (33)	3 (17)	-
Origin					
Acceptable	18	9 (50)	6 (33)	3 (17)	-
Labeling					
Acceptable	13	8 (62)	4 (31)	1 (7)	
Major	5	1 (20)	2 (40)	2 (40)	
Lot Identification					
Acceptable	17	9 (53)	5 (29)	3 (18)	1.0
Major	1	0 (0)	1 (100)	0 (0)	
Potential factors supporting survival of microbes, unsafe application of chemical in food					
Acceptable	11	7 (63.6)	2 (18)	2 (18)	0.5
Major problem	7	2 (28)	4 (57)	1 (14)	
Potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants during preparation					
Acceptable	10	3 (30)	4 (40)	3 (30)	0.05
Major and Serious problem	8	6 (75)	2 (25)	0 (0)	
Defrosting					

Acceptable	16	8 (50)	5 (31)	3 (19)	1.00
Major problem	2	1 (50)	1 (50)	0 (0)	

Cooling/ Heating

Acceptable	16	8 (50)	6 (37)	2 (13)	0.6
Major problem	2	1 (50)	0 (0)	1 (50)	

Cleaning/ Sanitation

Acceptable	8	5 (62)	2 (25)	1 (12)	0. 5
Major problem	10	4 (40)	4 (40)	2 (20)	

Hand washing and behavior of people in food establishment

Acceptable	13	6 (46)	6 (46)	1 (8)	0.7
Major problem	5	3 (60)	0 (0)	2 (40)	

Presence of adequate hand washing facilities

Acceptable	12	4 (33)	6 (50)	2 (16)	0.3
Major problem	6	5 (83)	0 (0)	1 (16)	

Dressing

Acceptable	15	7 (47)	6 (40)	2 (13)	
Major problem	3	2 (67)	0 (0)	1 (33)	

State of apparent injuries and health

Acceptable	18	9 (50)	6 (33)	3 (17)	-

Movement of people in the food establishment

Acceptable	18	9 (50)	6 (33)	3 (17)	-

Cleanliness

Acceptable	4	2 (50)	1 (25)	1 (25)	1.0
Major problem	14	7 (50)	5 (35)	2 (14)	

State, nature, design, use and operation of equipment

Acceptable	12	8 (66)	2 (16.7)	2 (16)	0.3
Major problem	6	1 (16)	4 (66)	1 (16)	

Presence of animals, insects and excreta

Acceptable	17	8 (47)	6 (35)	3 (17)	0.7
Major problem	1	1 (100)	0 (0)	0 (0)	

Environmental sources of contamination (chemical, physical or microbiological)

Acceptable	4	2 (50)	0 (0)	2 (50)	0.5
Major problem	14	7 (50)	6 (42)	1 (7)	

Water Supply (hot and cold)

Acceptable	17	8 (47)	6 (35)	3 (18)	0.7
Major problem	1	1 (100)	0 (0)	0 (0)	

* The exact Mantel–Haenszel (MH) Chi-square test was used to estimate the p-values

Table 4: Controls points scores from 18 establishments and relationship with E. coli and TAC

Inspector evaluation of control points		E. coli		TAC	
		n (%)	p-value	Mean	p-value
Ambient Temperature					
Acceptable	15	4 (27)	0.04	5.3	0.95
Major problem	3	3 (100)		5.3	
Internal temperature of the food					
Acceptable	16	5 (31)	0.1	5.4	0.51
Serious Problem	2	2 (100)		4.8	
Safety					
Acceptable	18	7 (39)	-	-	-
Origin					
Acceptable	18	7(39)	-	-	-
Labeling					
Acceptable	13	4 (31)	0.3	5.5	0.5
Major	5	3 (60)		5.1	
Lot Identification					
Acceptable	17	7 (41)	1.0	5.2	-
Major	1	0 (0)		6.5	
Potential factors supporting survival of microbes, unsafe application of chemical in food					
Acceptable	11	4 (36)	1.0	5.9	0.2
Major problem	7	3 (43)		5.6	
Potential factors supporting introduction of pathogenic microbes, toxic(hazardous) contaminants during preparation					
Acceptable	10	3 (30)	0.6	5.4	0.4
Major and serious problem	8	4 (50)		5.1	
Defrosting					
Acceptable	16	6 (10)	1.0	5.3	0.9
Major	2	1 (50)		5.3	
Cooling/Heating					
Acceptable	16	6 (10)	1.00	5.3	0.6
Major	2	1 (50)		4.9	
Cleaning/ Sanitation					
Acceptable	8	5 (62)	0.1	5.2	0.8
Major problem	10	2 (20)		5.3	
Hand washing and behavior of people in the food establishment					
Acceptable	13	5 (38)	1.0	5.5	0.1
Major	5	2 (40)		4.9	
Presence of adequate hand washing facilities					

	N	E. coli positive (%)	p*	TAC	p*
Acceptable	12	3 (25)	0.1	5.6	0.01
Major problem	6	4 (67)		4.7	
Dressing					
Acceptable	15	5 (33)	0.5	5.5	0.06
Major	3	2 (67)		4.4	
State of apparent health and injuries					
Acceptable	18	7 (39)	-		-
Movement of People in the food Establishment					
Acceptable	18	7 (39)	-		-
Cleanliness					
Acceptable	4	3 (75)	0.2	5.5	0.6
Major problem	14	4 (29)		5.4	
State, nature, design, use and operation of equipment					
Acceptable	12	5 (47)	1.0	5.2	0.4
Major problem	6	2 (33)		5.5	
Presence of animals, insects and excreta					
Acceptable	17	7 (41)	1.0	5.2	-
Major problem	1	0 (0)		7.6	
Environmental sources of contamination (chemical, physical or microbiological)					
Acceptable	4	4 (100)	0.01	5.2	0.8
Major problem	14	3 (21)		5.3	
Water Supply (Hot and cold)					
Acceptable	17	7 (41)	1.0	5.3	-
Major	1	0 (0)		4.7	

* Exact Pearson Chi-square and the unequal variances t- test were used to estimate the p-values for *E. coli* and TAC respectively.

* -When the scores of the inspectors were only a single value, statistical test has not been performed due to lack of variation.

Table 5: Establishments with varied number of control points with score of serious and major problem and association with *L. monocytogenes*, *E. coli* and TAC

No. of control points with major and /Sever scores	Levels of *L. monocytogenes*			Total Estab.	*P-value*	*E. coli*		Total estab.	*P-value*	Log 10 TAC	Total estab.	*P-value*
	0	1	2			P	A			Mean		
<4	2	1	1	4	1.0	2	3	5	1.0	4.8	4	0.3
≥4	7	5	2	14		5	8	13		5.5	14	
Total	9 (50)	6 (33)	3 (17)	18		7 (39)	11 (61)	18		5.15		

*The exact Mantel–Haenszel (MH) Chi-square test, exact Pearson Chi-square test and the unequal variance t-test were used to estimate the p-values of *L. monocytogenes*, *E. coli* and TAC respectively.

CHAPTER 4. GENERAL DISCUSSION

The issue of food safety is a constant threat to public due to the illnesses and death that arise form consumption of unsafe food. It also negatively affects the economic development *(46)*. For these reasons, numerous efforts have been exerted to mitigate the undesired impact through monitoring of food-borne diseases and pathogens in a farm-to-fork approach and application of evidence-based risk assessments *(6)*. Advancing the diagnostic technique in the laboratory and field context, regulatory and inspection harmonization issues at the national and international level, development of food safety risk assessment models, and complete and routine implementation of HACCP at the production and processing level remain as challenges in addressing food safety *(91, 121)*. In the process of addressing these challenges, food producing establishments around the globe are using different methods aiming to improve the quality and safety of their products and production process as well mainly through safe food production chain to and compliance with regulatory and customer requirements *(37, 57)*. Microbial testing is an important approach to assess the soundness of the inspection model, to set standards in relation to cleaning and sanitation, to assess the likelihood of the occurrence of hazards and set limits within a food establishment *(64)*. Using such a microbial test, we performed pathogenic and indicator microorganisms testing in different RTE food producing establishments and tried to assess if we can find an association with selected control points of the MAPAQ inspection model.

4.1. Environmental Samples and Presence of *Listeria monocytogenes* in RTE food establishment

Product contact surfaces and the environment within the food establishments are important sources of *L. monocytogenes (78, 134)* and we also noticed in our findings that various FCS and NFCS harbored this pathogen. Environmental sampling of a retail establishments in the USA indicated 1,161 *L. monocytogenes* positive samples. Among these, the number of NFCS samples were125 and 26 FCS samples from the overall 151 (13.0%) *L. monocytogenes* positive samples collected from different sites *(117)*. Similar studies showed the level of *L. monocytogenes* on FCS and NFCS of two poultry and pork processing establishments in France and *L. monocytogenes* were 65 of 313 in plant A and 33 of 155 in plant B *(22)*. On another study conducted in beef processing plant in the USA, *L. monocytogenes* was detected in 21 of 148 (14.2%) product contact surfaces including floor and conveyor belts *(110)*. Similarly, in our study, most of the *L. monocytogenes* was detected from FCS where a possible contamination could occur. However, our findings and some from other studies vary in terms of *L. monocytogenes* positivity, which might be due to the study design, sample size, sampling methodology and laboratory techniques used. In addition, the physiological characteristics of the various *L. monocytogenes* strains, as related to the serotypes, biofilm production, environmental condition such as type of surface and pretreatment used affect the prevalence of this pathogen in the food processing environment *(130)*.

An important aspect of any survey that aims to correlate microbiological data to risk factors is the representativeness of sampling. As a basic rule, CFIA recommends selection of 5 FCS and 5 NFCS sampling sites to better assess the establishment's control program

(16). Nevertheless, we used, for logistic reasons, the minimum requirement of samples per establishment i.e. 5 samples per establishment, 3 FCS and 2 NFCS. It may, however, had an impact the likelihood to establish correlation between the presence of bacterial pathogens such as *L. monocytogenes* or *Salmonella* in our study. As described by different authors, environmental factors, including the type of facilities and equipment design, that may make it difficult to clean, have been identified as key contributors to promote the persistence of the pathogen. However, the exact mechanisms to this persistence in the environment of such facilities and on the equipment, are much less well understood *(36, 66, 70)*. Nevertheless, when collecting a smaller number of samples in the establishment, such as we have done in this study, it is strongly suggested to take it from sites with the highest likelihood of contamination; it ultimately increases the probability of detection of microbes *(16)*. It explains why we have selected floors, nearby drains, as one of the sampling site in our study in an attempt to reduce the impact or reduced number of samples by establishment.

4.2. Isolation and Identification of *Listeria monocytogenes*

Selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and hemolytic properties are the gold standard for identification. The emergence of biotech tools leads innovation of various molecular diagnostic tools that target specific genome or proteome of the organism *(44)*. In our study, a two-step enrichment method recommended by the Health Canada MFHPB-30 standard technique and parallel, multiplex *RTi-PCR* analysis, targeting the *prfA* and *prs* genes *(63, 69)* were conducted to identify and confirm *L. monocytogenes* from 2ml of broth following secondary enrichment. Dalmasso et al. (2014) tested presence of *L. monocytogenes* DNA from the first and second enrichment using RTi- PCR. For the samples that gave different

results, plating was significantly more sensitive for detection of positive samples than RTi-PCR from the first enrichment. However, in accordance with our findings, RTi-PCR detected higher positive samples than plating from the second enrichment *(29)*.

4.3. Analysis of Food Safety Indicator Microorganisms

Different studies have been conducted with the aim to identify food borne pathogens as linked to good hygienic practices. Many have used *E. coli* and aerobic bacteria as food safety hygiene indicator microbes *(54, 65, 116)*. The level of indicator microorganisms counts in FCS and NFCS in the food processing environment is linked to the likelihood of cross-contamination that arise from these sources *(127)*.

In comparison to our results for *E. coli* and TAC, Gounadaki et al. (2007) reported higher load of *Enterobacteriaceae* and total viable counts collected form specific sampling sites within the RTE meat processing environment of different small-scale facilities where samples were taken from 40 cm×40 cm areas of FCS and NFCS *(45)*. This might be due to the relative difference in the application of cleaning and sanitation procedures that affect the hygienic status of establishments at the time of sampling, which might in turn affect the overall load of microbes. In another study, a bacterial load of TAC (>3.9 log CFU/50 cm^2) was observed from samples collected from gloves of food handlers and on food contact surfaces *(65)*. These counts were higher than our own results in terms of presence of *E. coli*, suggesting punctual fecal contamination. On the other hand, the TAC load was smaller than our finding, suggesting a better efficacy of the overall hygienic measures taken in the processing plants. The limited number of sampling sites within the establishment, the limited number of sampled establishments and the convenient sampling

scheme used in the current study clearly affect the possibility to make inference to the general population of RTE establishment under MAPAQ jurisdiction as far as indicator microorganisms are concerned. It is certainly the case as well for pathogenic microorganisms.

4.4. Microbial Association with Control Points of the MAPAQ Inspection Model

The risk-based food inspection is one of the emerging tools used in modern food control systems. The application of independent microbial analysis, have been found to be supportive in assessing the performance of such inspection models *(58)*. Among selected control points in the MAPAQ inspection model, "ambient temperature", "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants", "environmental sources of contamination (chemical, physical or microbiological)", "presence of adequate hand washing facility", in RTE food establishments were associated with *L. monocytogenes*, indicator *E. coli* and total aerobic count.

In line with our own findings, "ambient temperature" and its positive impact on the presence and level of *L. monocytogenes* and *E. coli* in food processing establishments has been demonstrated by different studies *(5, 21, 53, 84, 95)*. It suggests that compliance to ambient temperature requirements could be used to indicate the likelihood of recovering *L. monocytogenes* within food establishments. It suggests also that this control point can be considered as a critical component of the development of an early warning system to prevent *Listeria monocytogenes* related illnesses.

The control point "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants" mainly identifies factors that result in cross contamination from utensils and equipment used in the food establishment. It also deals with use of certain ingredients that may have a toxic effect to the public. Use of common utensils and processing tables for a variety of food preparation at different stages of production and preparing different types of food without changing the cooking oil have been pointed out as some of the issues that enhance cross contamination *(87)*. In our study this control point has shown a significant association with the levels of *L. monocytogenes. L. monocytogenes* is commonly found in the food processing environment and its abundance in the environment could result in cross-contamination of different food contact surfaces and the final product as well *(134, 135)*. As it was observed in our study, the ambient temperature was also associated with the presence of this pathogen. It may also promote the growth and multiplication of the organism with in the food establishment environment that in turn results in cross contamination.

Possible "environmental sources of contaminants" including glass or wood shards, and uncleaned premises including processing tables and knifes, were found, in the RTE processing plants, to be positively associated with the presence of *E. coli*. This was supported by other studies that demonstrated a positive association between various FCS, such as cutting boards, grinders, knifes and the presence of *E. coli (115, 120)*. A Belgium study showed a link between QA activities and the presence and distribution of pathogenic bacteria in the poultry meat preparations. Identification of high load of microbes at the working tables could be an indication of insufficient sanitation measures (e.g., cleaning and disinfection program, hygienic design, and compliance with hygiene procedures). The prevalence and high counts of *E. coli* and *Enterobacteriaceae* were associated with the high initial levels in the carcasses (due to contaminated flocks on farms) and subsequent
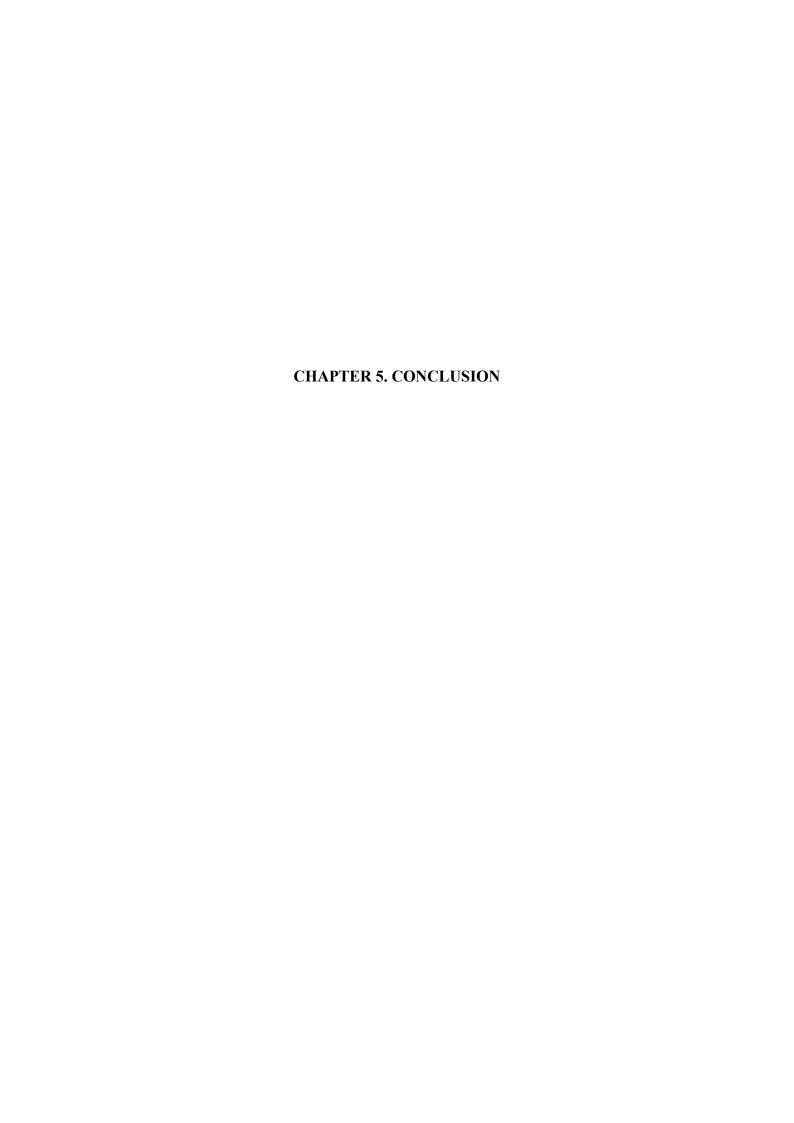
cross-contamination (e.g., via the workers, contact materials, tools, and equipment) during processing *(116)*. *E. coli*, being a member of the enterobacteria group, is present stool and its finding in the environment and FCS suggests fecal contamination of the product and ultimately of the processing area *(56)*. However, the control point "Cleaning/ Sanitation" in our finding did not show any significant association with the level of neither *L. monocytogenes* nor indicators, suggesting that good disinfection measures were applied within the establishments.

The safety of the food during the entire production process is highly dependent on the food handlers behavior and application of hygienic measures and hygienic food handling *(122)*. Some studies have shown the positive association between improper hand hygiene in retail and home food preparation and various pathogenic and indicator microbes *(9, 72)*. In accordance with this, we found a, positive association between the load of TAC ($p = 0.01$) and the control point "presence of adequate hand washing facilities" in meat related RTE. This suggests that a lack of adequate hand washing facilities in food processing plants eventually lead to a potential contamination of the product. Equipment and utensils used in the food production may become contaminated and become a source of cross-contamination by the different types of pathogenic and hygiene indicator like aerobic microbes *(122)*. To prevent any physical, chemical or biological contamination of the product, FCS of the equipment should be cleaned *(28)*.

In the present study, we used a convenient sampling during selection of RTE food producing establishment based on MAPAQ`s pre-planned regular and follow-up inspection schedule and preferred geographical location (South shore of Montréal was selected considering logistic and distance from the laboratory facility). This type of sampling
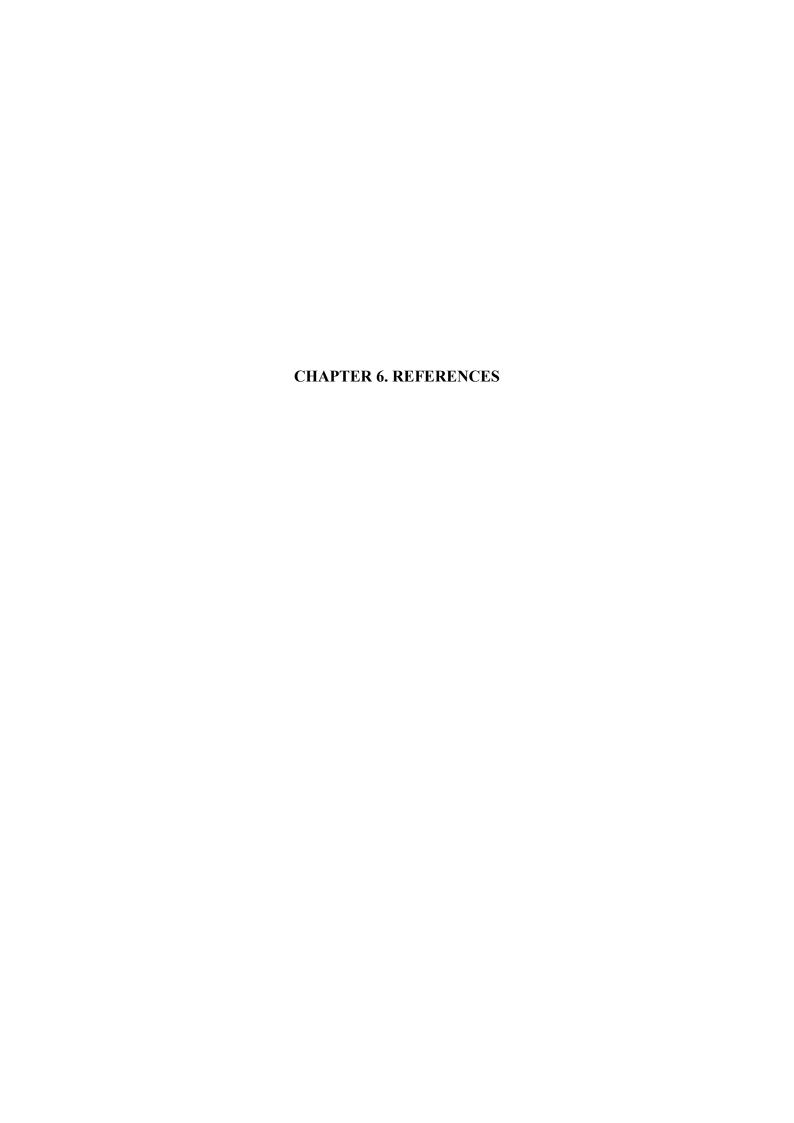
technique and study results are not necessarily representative of the population *(124)* and we cannot infer from our findings to the entire RTE producing establishments located in Quebec. This could generate a selection bias *(55)*. Additionally, the study areas were very limited. As a matter of consequence, this study could be considered as a preliminary report to design further studies using a probabilistic sampling in different parts of the province.

Compared with the huge number of food establishment located in the province of Quebec, our sample size was indeed relatively small. Increasing the sample size in our study could have increase the statistical power *(55)* that eventually strengthen the outcome of association estimation between the control points and microorganisms in all food establishments i.e. external populations. However, due to the nature of our sampling strategy, the control points and microbial association found in our study can only be extrapolated to similar food establishments located in the study area *(55)*. Nevertheless, the positive association found in this study, particularly those associated with *L. monocytogenesis*, can be of great value for those that would like to design surveillance or preventive program targeting this microorganism.

CHAPTER 5. CONCLUSION

The Quebec risk based food inspection system comprises of checklist that summarizes the different aspects of control points in food establishments. The present preliminary study provides evidences on the association of some control points from the MAPAQ inspection model, namely "ambient temperature", "potential factors supporting introduction of pathogenic microbes, toxic (hazardous) contaminants", "environmental sources of contamination (chemical, physical or microbiological)", and "presence of adequate hand washing facility" with *L. monocytogenes* and indicator *E. coli* and total aerobic count. Besides this, our study indicated a regular presence of *L. monocytogenes* in various FCS and NFCS in RTE food producing establishments, which could result cross contamination which occurs when RTE product directly contacts a surface that has been contaminated with *L. monocytogenes*. To prevent cross-contamination, establishments need to ensure that sanitation is effectively maintained, with special attention being given to those areas where product is stored or handled after a lethality treatment has been applied to the product. It is important to underline the fact that very few of the establishments that participated in the current research project possessed a full HACCP plan since they are under the jurisdiction of MAPAQ, the provincial authority responsible for inspecting those facilities.

Using the information from our current study as a baseline, further studies should be conducted with a representative from the different areas of Quebec so that it will be possible to extrapolate it at a population level and to confirm the observed association between control points and pathogenic and indicator microbes. However, one should consider using the positive association found in the current study between *L. moncytogenes* and some inspection control points in the design of preventive strategies against this bacterium.

CHAPTER 6. REFERENCES

1. Abelson P., F. M. Poter and H. Gillian. 2006. The annual cost of foodborneillness in Australia. Department of Health and Ageing, Canberra.

2. Abubakar I., L. Irvine, C. F. Aldus, G. M. Wyatt, R. Fordham, S. Schelenz, L. Shepstone, A. Howe, M. Peck, and P. R. Hunter. 2007. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol Assess.*11:1-216.

3. Annie L., M. Hatcher, and A. Papadopoulos. 2014. Risk Factors and Surveillance Systems for Foodborne Illness Outbreaks in Canada. Available at: http://www.ncceh.ca/sites/default/files/Risk_Surveillance_FBI_Outbreaks_June_2014.pdf. Accessed 13 July, 2016.

4. Aparecida de O. M., E. G. Abeid Ribeiro, A. M. Morato Bergamini, and E. C. Pereira De Martinis. 2010. Quantification of Listeria monocytogenes in minimally processed leafy vegetables using a combined method based on enrichment and 16S rRNA real-time PCR. *Food Microbiol.* 27:19-23.

5. Aureli P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by Listeria monocytogenes. *N Engl J Med.* 342:1236-41.

6. Batz M. B., M. P. Doyle, G. Morris, Jr., J. Painter, R. Singh, R. V. Tauxe, M. R. Taylor, and D. M. Lo Fo Wong. 2005. Attributing illness to food. *Emerging Infect. Dis.* 11:993-9.

7. Beuchat L. R., and J. H. Ryu. 1997. Produce handling and processing practices. *Emerging Infect. Dis.* 3:459-65.

8. Bhagwat A. A., J. Patel, T. Chua, A. Chan, S. R. Cruz, and G. A. Aguilar. 2008. Detection of Salmonella species in foodstuffs. *Methods Mol. Biol.*429:33-43.

9. Borrusso P. A., and J. J. Quinlan. 2017. Prevalence of Pathogens and Indicator Organisms in Home Kitchens and Correlation with Unsafe Food Handling Practices and Conditions. *J.Food Prot.80*:590-597.

10. Brenner F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. Salmonella nomenclature. *J. Clin. Microbiol.* 38:2465-7.

11. Bryan Frank L. 1988. Risks of Practices, Procedures and Processes that Lead to Outbreaks of Foodborne Diseases. *J.Food Prot. 51*: 663-673.

12. Buchanan R. L. and R. Oni. 2012. Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. *J.Food Prot.* 75(5):989-997.

13. Butler A. J., M. K. Thomas, and K. D. Pintar. 2015. Expert elicitation as a means to attribute 28 enteric pathogens to foodborne, waterborne, animal contact, and person-to-person transmission routes in Canada. *Foodborne Pathog. Dis.*12:335-44.

14. Buzby J. C., and T. Roberts. 2009. The economics of enteric infections: human foodborne disease costs. *Gastroenterology*. 136:1851-62.

15. Canadian Food Inspection Agency. 2013. Causes of Food Poisoning. Available at: http://www.inspection.gc.ca/food/information-for-consumers/fact-sheets-and-infographics/food-poisoning/eng/1331151916451/1331152055552. Accessed 12 January 2017.

16. Canadian Food Inspection Agency. 2013. Environmental and Finished Dairy Product Sampling Monitoring for Listeria monocytogenes. Available at: http://www.inspection.gc.ca/food/dairy-products/manuals-inspection-procedures/product-inspection/monitoring-for-listeria-monocytogenes/eng/1335465632611/1335466303763?chap=9. Accessed 27 August 2016.

17. Canadian Food Inspection Agency. 2016. Food complaints, food safety investigations and food recalls. Available at: http://www.inspection.gc.ca/about-the-cfia/newsroom/food-safety-system/eng/1332207100013/1332207173484. Accessed 4 May 2016.

18. Canadian Food Inspection Agency. 2017. Federally registered food establishments located in Quebec.

19. Canadian Public Health Association. 2016. Who is responsible for food safety in Canada? Available at: http://www.cpha.ca/en/programs/history/achievements/09-shf/safety.aspx. Accessed June 23, 2016.

20. Carol A., W.L. Holyoakb, S.C. Powellc, and F.C. Dykesd. 2012. Re-thinking the HACCP team: An investigation into HACCP team knowledge and decision-making for successful HACCP development. *Food Res Int*. 47:236-245.

21. Castro-Ibanez I., M. I. Gil, J. A. Tudela, R. Ivanek, and A. Allende. 2015. Assessment of microbial risk factors and impact of meteorological conditions during production of baby spinach in the Southeast of Spain. *Food Microbiol*. 49:173-81.

22. Chasseignaux E., M. T. Toquin, C. Ragimbeau, G. Salvat, P. Colin, and G. Ermel. 2001. Molecular epidemiology of Listeria monocytogenes isolates collected from the environment, raw meat and raw products in two poultry- and pork-processing plants. *J. Appl. Microbiol.*. 91:888-99.

23. Cheng C. M., W. Lin, K. T. Van, L. Phan, N. N. Tran, and D. Farmer. 2008. Rapid detection of Salmonella in foods using real-time PCR. *J.Food Prot*.71:2436-2441.

24. Clayton D. A., C. J. Griffith, P. Price, and A. C. Peters. 2002. Food handlers' beliefs and self-reported practices. *Int J Environ Health Res*. 12:25-39.

25. Codex Alimentarius Commission. 2008. Guidelines for the validation of food safety control measures Available at: www.fao.org/input/download/standards/11022/CXG_069e.pdf. Accessed 26 April, 2017.

26. Codex Alimentarius Commission. 2013. Principles and Guidelines for National Food Control Sstems. Available at: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BGL%2B82-2013%252FCXG_082e.pdf. Accessed 26 July 2016.

27. Cruickshank J. G. 1990. Food handlers and food poisoning. *BMJ*. 300:207-8.

28. Dunsmore D. G., A. Womey, W. G. Whittlestone and H. W. Morgan. 1981. Design and Performance of Systems for Cleaning Product-Contact Surfaces of Food Equipment: A Review. *J.Food Prot*. 44: 220-240.

29. Dalmasso M., A. S. Bolocan, M. Hernandez, A. E. Kapetanakou, T. Kuchta, S. G. Manios, B. Melero, J. Minarovicova, M. Muhterem, A. I. Nicolau, J. Rovira, P. N. Skandamis, B. Stessl, M. Wagner, K. Jordan, and D. Rodriguez-Lazaro. 2014. Comparison of polymerase chain reaction methods and plating for analysis of enriched cultures of Listeria monocytogenes when using the ISO11290-1 method. *J. Microbiol. Methods*. 98:8-14.

30. Dennis Sherri B., B.L. Robert, M.J. Arthur. 2002. Microbial risk assessment: Achievements and future challenges. Available at: http://vm. cfsan. fda. gov/~ acrobat/qmrisk. pdf. Accessed 21 January 2017.

31. Dhama K., S. Rajagunalan, S. Chakraborty, A. K. Verma, A. Kumar, R. Tiwari, and S. Kapoor. 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pak. J. Biol. Sci*.16:1076-85.

32. Douglas A., J. Powell A Casey, J. Jacobs, and B. J. Chapmanb. 2011. Enhancing food safety culture to reduce rates of foodborne illness. *Food Control*. 22: 817-822.

33.Doménecha E., I. Escrichea, and S. Martorellb. 2008. Assessing the effectiveness of critical control points to guarantee food safety. *Food Control*. 19: 557–565.

34.European Comission. 2002. Risk Assessment of Food borne Bacterial Pathogens. Available at: http://ec.europa.eu/food/fs/sc/ssc/out252_en.pdf. Accessed 11 February 2015.

35.European Comission. 2017. Microbiological criteria. Available at: https://ec.europa.eu/food/safety/biosafety/food_hygiene/microbiologic al_criteria_en. Accessed 26 April 2017.

36.Ferreira V., M. Wiedmann, P. Teixeira, and M. J. Stasiewicz. 2014. Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *J.Food Prot*. 77:150-70.

37.Food and Agriculture Organization. 2003. Assuring Food Safety and Quality: Guidelines for Strengthening National Food Control Systems. Available at: ftp://ftp.fao.org/docrep/fao/006/y8705e/y8705e00.pdf. Accessed 27 April 2016.

38.Food and Agriculture Organization. 2005. The Food Safety Regulatory System in Canada. Available at: http://www.fao.org/3/a-af190e.pdf. Accessed 5 May 2016.

39.Food and Agriculture Organization. 2005. Food Safety Risk Analysis - Part I - An Overview and Framework Manual - Provisional Edition. Available at: https://www.fsc.go.jp/sonota/foodsafety_riskanalysis.pdf. Accessed 6 February 2016.

40.Food and Agriculture Organization. 2007. Strengthening national food control systems. A quick guide to assess capacity building needs Available at: http://www.fao.org/3/a-a1142e.pdf. Accessed 27 April 2016.

41.Food and Agriculture Organization. 2008. Risk-based food inspection manual. Available at: http://www.fao.org/3/a-i0096e.pdf. Accessed 13 April 2016.

42.Food and Agriculture Organization. 2011. Preventing E. coli in Food. Available at: www.fao.org/food-chain-crisis/resources/news/detail/en/c/80884/. Accessed 28 June 2016.

43.Freitas N.O.D., RAC.Penha Filho, P. Barrow, and B. Junior 2010. Sources of Human Non-Typhoid Salmonellosis: A Review. *Rev. Bras. Cienc. Avic*. 12:01-11.

44.Gasanov U., D. Hughes, and P. M. Hansbro. 2005. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. *FEMS Microbiol. Rev*.29:851-75.

45. Gounadaki A. S., P. N. Skandamis, E. H. Drosinos, and G. J. Nychas. 2008. Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. *Food Microbiol.* 25:313-23.

46. Havelaar A. H., S. Brul, A. de Jong, R. de Jonge, M. H. Zwietering, and B. H. Ter Kuile. 2010. Future Challenges to Microbial Food Safety. *Int. J. Food Microbiol.* 139:79-94.

47. Havelaar A. H., M. D. Kirk, P. R. Torgerson, H. J. Gibb, T. Hald, R. J. Lake, N. Praet, D. C. Bellinger, N. R. de Silva, N. Gargouri, N. Speybroeck, A. Cawthorne, C. Mathers, C. Stein, F. J. Angulo, and B. Devleesschauwer. 2015. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Med..* 12:1001923.

48. Health Canada. 2007. Risk Categorization Model for Food Retail /Food Service Establishments Available at: http://www.hc-sc.gc.ca/ahc-asc/alt_formats/hpfb-dgpsa/pdf/pubs/risk_categorization-categorisation_risques-revised_revisee-eng.pdf. Accessed 12 July 2016.

49. Health Canada. 2013. Microbial Guidelines for Ready-to-Eat Foods – A Guide for the Conveyance Industry and Environmental Health Officers (EHO). Available at: http://publications.gc.ca/collections/collection_2014/sc-hc/H164-167-2013-eng.pdf. Accessed 28 June 2016.

50. Hedberg C. W., K. E. White, J. A. Johnson, L. M. Edmonson, J. T. Soler, J. A. Korlath, L. S. Theurer, K. L. MacDonald, and M. T. Osterholm. 1991. An outbreak of Salmonella enteritidis infection at a fast-food restaurant: implications for foodhandler-associated transmission. *J. Infect. Dis.* 164:1135-40.

51. Herikstad H., Y. Motarjemi, and R. V. Tauxe. 2002. Salmonella surveillance: a global survey of public health serotyping. *Epidemiol. Infect.* 129:1-8.

52. Hoelzer K., A. I. Moreno Switt, and M. Wiedmann. 2011. Animal contact as a source of human non-typhoidal salmonellosis. *Vet Res.* 42:34.

53. Huis in 't Veld J. H. 1996. Microbial and biochemical spoilage of foods: an overview. *Int. J. Food Microbiol..* 33:1-18.

54. Hutchison M. L., L. D. Walters, G. C. Mead, M. Howell, and V. M. Allen. 2006. An assessment of sampling methods and microbiological hygiene indicators for process verification in poultry slaughterhouses. *J.Food Prot.* 69:145-53.

55. Ian D., W.Martin, and H.Stryhn. 2003. Veterinary Epidemiologic Research. University of Prince Edward Island, Prince Edward Island, Canada.

56. International Commission on Microbiological Specifications for Foods (ICMSF). 1988. *Microorganisms in foods 1: their significance and methods of enumeration.* University of Toronto Press, Toronto.

57. Jacques T., and P. Zuurbier. 2008. Quality and safety standards in the food industry, developments and challenges. *Int J Prod Econ*. 113:107-122.

58. Jacxsens L., J. Kussaga, P. A. Luning, M. Van der Spiegel, F. Devlieghere, and M. Uyttendaele. 2009. A Microbial Assessment Scheme to measure microbial performance of Food Safety Management Systems. *Int. J. Food Microbiol.*. 134:113-25.

59. Jacxsens L., M. Uyttendaele, F. Devlieghere, J. Rovira, S. O. Gomez, and P. A. Luning. 2010. Food safety performance indicators to benchmark food safety output of food safety management systems. *Int. J. Food Microbiol.*. 141 Suppl 1:S180-7.

60. Jemmi T., and R. Stephan. 2006. Listeria monocytogenes: food-borne pathogen and hygiene indicator. *Rev. - Off. Int. Epizoot.* 25:571-80.

61. Jennifer L.J. 1996. Predictive Microorganisms as an Indication of Pathogen Contamination. p. 6. *In*, American Meat Science Association, vol. 49. St., Madison, WI.

62. Katleen B., X.V. Huffel, O. Wilmart, L. Jacxsens, D. Berkvens, H. Diricks, A. Huyghebaert, and M. Uyttendaele. 2011. Measuring the safety of the food chain in Belgium: Development of a barometer. *Food Res Int*. 44:940–950.

63. Kerouanton A., M. Marault, L. Petit, J. Grout, T. T. Dao, and A. Brisabois. 2010. Evaluation of a multiplex PCR assay as an alternative method for Listeria monocytogenes serotyping. *J. Microbiol. Methods*. 80:134-7.

64. Kvenberg J. E., and D. J. Schwalm. 2000. Use of microbial data for hazard analysis and critical control point verification--Food and Drug Administration perspective. *J.Food Prot*. 63:810-4.

65. Lahou E., L. Jacxsens, J. Daelman, F. Van Landeghem, and M. Uyttendaele. 2012. Microbiological performance of a food safety management system in a food service operation. *J.Food Prot.* 75:706-16.

66. Lambertz S. T., C. Nilsson, A. Bradenmark, S. Sylven, A. Johansson, L. M. Jansson, and M. Lindblad. 2012. Prevalence and level of Listeria monocytogenes in ready-to-eat foods in Sweden 2010. *Int. J. Food Microbiol.*. 160:24-31.

67. Lammerding A. M., and A. Fazil. 2000. Hazard Identification and Exposure Assessment for Microbial Food Safety Risk Assessment. *Int. J. Food Microbiol.* 58:147-57.

68. Lammerding Anna M. 1997. An Overview of Microbial Food Safety Risk Assessment. *J.Food Prot.*:1302-1471.

69. Lariviere-Gauthier G., A. Letellier, A. Kerouanton, S. Bekal, S. Quessy, S. Fournaise, and P. Fravalo. 2014. Analysis of Listeria monocytogenes strain distribution in a pork slaughter and cutting plant in the province of Quebec. *J Food Prot*. 77:2121-8.

70. Lianou A., and J. N. Sofos. 2007. A review of the incidence and transmission of Listeria monocytogenes in ready-to-eat products in retail and food service environments. *J. Food Prot.* 70:2172-98.

71. Lillquist D. R., M. L. McCabe, and K. H. Church. 2005. A comparison of traditional handwashing training with active handwashing training in the food handler industry. *J Environ Health.* 67:13-6, 28.

72. Lues J.F.R. and I.V.Tonder. 2007. The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. *Food Control*. 18: 326-332.

73. Brown M.H., C.O. Gill, J. Hollingsworth, R. Nickelson, S. Seward, J.J. Sheridan, T. Stevenson, J.L. Sumner, D.M. Theno, W.R. Usborne, and D. Zink. 2000. The role of microbiological testing in systems for assuring the safety of beef. *Int. J. Food Microbiol..* 62:7–16.

74. Malik A.Hussain and Christopher O. Dawson. 2013. Economic Impact of Food Safety Outbreaks on Food Businesse. *Foods* 2:585-589.

75. Malorny B., C. Lofstrom, M. Wagner, N. Kramer, and J. Hoorfar. 2008. Enumeration of salmonella bacteria in food and feed samples by real-time PCR for quantitative microbial risk assessment. *Appl Environ Microbiol.* 74:1299-304.

76. Malorny B., E. Paccassoni, P. Fach, C. Bunge, A. Martin, and R. Helmuth. 2004. Diagnostic real-time PCR for detection of Salmonella in food. *Appl. Environ. Microbiol..* 70:7046-52.

77. MAPAQ. 2017. Standarde refernces used for MAPAQ model development and number of establishments inispected under it`s jurisdiction.

78. Martin B., A. Perich, D. Gomez, J. Yanguela, A. Rodriguez, M. Garriga, and T. Aymerich. 2014. Diversity and distribution of Listeria monocytogenes in meat processing plants. *Food Microbiol.* 44:119-27.

79. McCabe-Sellers B. J., and S. E. Beattie. 2004. Food safety: emerging trends in foodborne illness surveillance and prevention. *J Am Diet Assoc.* 104:1708-17.

80. McLauchlin J., R. T. Mitchell, W. J. Smerdon, and K. Jewell. 2004. Listeria monocytogenes and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int. J. Food Microbiol..* 92:15-33.

81. McWhorter A. R., D. Davos, and K. K. Chousalkar. 2015. Pathogenicity of Salmonella strains isolated from egg shells and the layer farm environment in australia. *Appl. Environ. Microbiol..* 81:405-14.

82. Medeiros L. C., V. N. Hillers, P. A. Kendall, and A. Mason. 2001. Food safety education: what should we be teaching to consumers? *Journal of nutrition education*. 33:108-13.

83. Medeiros L., V. Hillers, P. Kendall, and A. Mason. 2001. Evaluation of food safety education for consumers. *J Nutr Educ.* 33 Suppl 1:S27-34.

84. Membre J. M., M. Kubaczka, J. Dubois, and C. Chene. 2004. Temperature effect on Listeria monocytogenes growth in the event of contamination of cooked pork products. *J.Food Prot.* 67:463-9.

85. Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec (MAPAQ). 2015. Bonnes pratiques d'hygiène et de salubrité alimentaires Étiquetage. Available at: http://www.mapaq.gouv.qc.ca/fr/Restauration/Qualitedesaliments/securitealiments/inspection/methodeinspection/Pages/bonnespratiqueshygienesalubrite.aspx. Accessed 10 March, 2017.

86. Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec. 2013. Guide de'Application de la Méthod D'Inspectiopn Basée Sur le Risque. Available at: http://www.mapaq.gouv.qc.ca/fr/Restauration/Qualitedesaliments/securitealiments/inspection/methodeinspection/Pages/bonnespratiqueshygienesalubrite.aspx. Accessed 15 February, 2015.

87. Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec (MAPAQ). 2017. Bonnes pratiques d'hygiène et de salubrité alimentaires. Available at: http://www.mapaq.gouv.qc.ca/fr/Restauration/Qualitedesaliments/securitealiments/inspection/methodeinspection/Pages/bonnespratiqueshygienesalubrite.aspx. Accessed 10 March, 2017.

88. Munro D., J.C. Le Vallée, J.Stuckey. 2012. Improving Food Safety in Canada: Toward a More Risk-Responsive System. Available at: http://www.conferenceboard.ca/cfic/research/2012/improvingfoodsafety.aspx. Accessed 12 August 2016.

89. National Institute of Health. 2012. Foodborne Illnesses Available at: www.digestive.niddk.nih.gov. . Accessed 11 January, 2016.

90. Nayak D. N., C. V. Savalia, I. H. Kalyani, R. Kumar, and D. P. Kshirsagar. 2015. Isolation, identification, and characterization of Listeria spp. from various animal origin foods. *Vet World.* 8:695-701.

91. Newell D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. van der Giessen, and H. Kruse. 2010. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.*. 139 Suppl 1:S3-15.

92. Norton D.M. 2002. Polymerase Chain Reaction-Based Methods for Detection of Listeria monocytogenes: Toward Real-Time Screening

for Food and Environmental Samples. *Journal of AOAC International.* 85:505-515.

93. O'Grady J., M. Ruttledge, S. Sedano-Balbas, T. J. Smith, T. Barry, and M. Maher. 2009. Rapid detection of Listeria monocytogenes in food using culture enrichment combined with real-time PCR. *Food Microbiol.* 26:4-7.

94. Olaimat A. N. and R. A. Holley. 2012. Factors influencing the microbial safety of fresh produce: a review. *Food Microbiol..* 32:1-19.

95. Oses S. M., P. A. Luning, L. Jacxsens, S. Santillana, I. Jaime, and J. Rovira. 2012. Microbial performance of food safety management systems implemented in the lamb production chain. *J Food Prot.* 75:95-103.

96. Luning P.A., K. Kirezieva, G. Hagelaar, J. Rovira, M. Uyttendaele, and L. Jacxsens. 2015. Performance assessment of food safety management systems in animal-based food companies in view of their context characteristics: A European study. *Food Control.* 49:11-22.

97. Luninga P.A., W.J. Marcelis, J. Rovira, M. Van der Spiegel, M. Uyttendaele, L. Jacxsens. 2009. Systematic assessment of core assurance activities in a company specific food safety management system. *Trends Food Sci Technol.* 20:300–312.

98. Panisello P. J., R. Rooney, P. C. Quantick, and R. Stanwell-Smith. 2000. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *Int. J. Food Microbiol..* 59:221-34.

99. Peccio A., T. Autio, H. Korkeala, R. Rosmini, and M. Trevisani. 2003. Listeria monocytogenes occurrence and characterization in meat-producing plants. *Lett Appl Microbiol.* 37:234-8.

100. Porwollik S., E. F. Boyd, C. Choy, P. Cheng, L. Florea, E. Proctor, and M. McClelland. 2004. Characterization of Salmonella enterica subspecies I genovars by use of microarrays. *J Bacteriol.* 186:5883-98.

101. Public Health Agency of Canada. 2011. Salmonella enterica spp. Pathogen Safety Data Sheet - Infectious substances. Available at: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/salmonella-ent-eng.php#note12. Accessed 24 June 2016.

102. Public Health Agency of Canada. 2012. Lessons Learned Review: Public Health Agency of Canada and Health Canada Response to the 2008 Listeriosis Outbreak. Available at: http://www.phac-aspc.gc.ca/fs-sa/listeria/2008-lessons-lecons-eng.php. Accessed 12 April, 2015.

103. Public Health Agency of Canada. 2013. Canada's Food-borne Illness Outbreak Response Protocol (FIORP) 2010: To guide a multi-jurisdictional response. Available at: http://www.phac-

aspc.gc.ca/zoono/fiorp-mitioa/index-eng.php#toc6_1. Accessed 7March 2017.

104. Public Health Agency of Canada. 2013. Food-borne and Water-borne Infections—Invisible Threats. Available at: http://www.phac-aspc.gc.ca/cphorsphc-respcacsp/2013/food-water_alim-eau-eng.php#table-1. Accessed 12 January, 2017.

105. Public Health Agency of Canada. 2014. Estimating the burden of food-borne illness in Canada. Available at: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/14vol40/dr-rm40-14/dr-rm40-14-comm-eng.php#footnotei. Accessed 13 January 2017.

106. Public Health Agency of Canada. 2016. Salmonella fact sheet. Available at: http://www.phac-aspc.gc.ca/fs-sa/fs-fi/salmonella-eng.php. Accessed 15 August 2016.

107. Public Health Agency of Canada. 2016. Yearly food-borne illness estimates for Canada. Available at: http://healthycanadians.gc.ca/eating-nutrition/risks-recalls-rappels-risques/surveillance/illness-estimates-estimations-maladies/yearly-annuel-eng.php. Accessed June 12, 2016.

108. Reij M. W., and E. D. Den Aantrekker. 2004. Recontamination as a source of pathogens in processed foods. *Int. J. Food Microbiol.*. 91:1-11.

109. Risk Assessment Technical Committee. Canadian Food Inspection Agency. 2014. Risk Assessment Model

110. Rivera-Betancourt M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella in two geographically distant commercial beef processing plants in the United States. *J.Food Prot.*. 67:295-302.

111. Robinson A. L., H. J. Lee, J. Kwon, E. Todd, F. P. Rodriguez, and D. Ryu. 2016. Adequate Hand Washing and Glove Use Are Necessary To Reduce Cross-Contamination from Hands with High Bacterial Loads. *J Food Prot.* 79:304-8.

112. Rocourt J., G. Moy, K. Vierk, and J. Schlundt. 2003. The present state of foodborne disease in OECD countries. Available at: http://www.who.int/foodsafety/publications/foodborne_disease/oecd_fbd.pdf. Accessed 12 January, 2016.

113. Rooney R. M., E. H. Cramer, S. Mantha, G. Nichols, J. K. Bartram, J. M. Farber, and P. K. Benembarek. 2004. A Review of Outbreaks of Foodborne Disease Associated with Passenger Ships: Evidence for Risk Management. *Public health reports (Washington, D.C. : 1974)*. 119:427-34.

114.Ross C.B., D.P. Suresh, D.P. Timothy, and L.Z.Richard. 2004. *Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions.* Institute of Food Technologists and Blackwell.

115.Sagoo S. K., C. L. Little, C. J. Griffith, and R. T. Mitchell. 2003. Study of cleaning standards and practices in food premises in the United Kingdom. *Commun. Dis. Public Health*. 6:6-17.

116.Sampers I., L. Jacxsens, P. A. Luning, W. J. Marcelis, A. Dumoulin, and M. Uyttendaele. 2010. Performance of food safety management systems in poultry meat preparation processing plants in relation to Campylobacter spp. contamination. *J.Food Prot.*. 73:1447-57.

117.Sauders B. D., M. D. Sanchez, D. H. Rice, J. Corby, S. Stich, E. D. Fortes, S. E. Roof, and M. Wiedmann. 2009. Prevalence and molecular diversity of Listeria monocytogenes in retail establishments. *J.Food Prot.*. 72:2337-49.

118.Schlech W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis-evidence for transmission by food. *The N. Engl. J. Med.*. 308:203-6.

119.Shabbir Simjee (ed.). 2007. *Foodborne Diseases.* Humana Press Inc., Totowa.

120.Silva D. A., M. R. Dias, M. V. Cossi, N. P. Castilho, A. C. Camargo, and L. A. Nero. 2016. Hygiene and Safety in the Meat Processing Environment from Butcher Shops: Microbiological Contamination and Listeria monocytogenes. *J.Food Prot.* 79:628-34.

121.Sofos J. N. 2008. Challenges to meat safety in the 21st century. *Meat science*. 78:3-13.

122.Sousa Cristina Paiva de. 2008. The Impact of Food Manufacturing Practices on Food borne Diseases *Braz Arch Biol Technol*. 51

123.Su L. H., and C. H. Chiu. 2007. Salmonella: clinical importance and evolution of nomenclature. *Chang Gung medical journal*. 30:210-9.

124.Suen L. J., H. M. Huang, and H. H. Lee. 2014. A comparison of convenience sampling and purposive sampling. *Hu li za zhi The Journal of nursing*. 61:105-11.

125.Swanson K. M. and J. E Anderson,. 2000. Industry perspectives on the use of microbial data for hazard analysis and critical control point validation and verification. *J.Food Prot.* 63:815-8.

126.Swartz M. N. 2002. Human Diseases Caused by Foodborne Pathogens of Animal Origin. *Clin. Infect. Dis.: an official publication of the Infectious Diseases Society of America*. 34 S111-22.

127.Syne S. M., A. Ramsubhag, and A. A. Adesiyun. 2013. Microbiological hazard analysis of ready-to-eat meats processed at a food plant in Trinidad, West Indies. *Infect Ecol Epidemiol*. 3.

128. The Australia New Zealand Food Authority. 2001. Food Safety:The priority classification system for food businesses. Available at: http://www.foodstandards.gov.au/publications/documents/ANZFA_15 78_Info_Paper__final.pdf. Accessed 10anuary, 2016.

129. The Centre for Food Safety Food and Environmental Hygiene Department of Hong Kong. 2007. Microbiological Guidelines for Ready-to-eat Food Available at: http://blpd.dss.go.th/micro/ready.pdf. Accessed 25 April 2015.

130. Thevenot D., A. Dernburg, and C. Vernozy-Rozand. 2006. An updated review of Listeria monocytogenes in the pork meat industry and its products. *J. Appl. Microbiol.* 101:7-17.

131. Thomas M. K., R. Murray, L. Flockhart, K. Pintar, A. Fazil, A. Nesbitt, B. Marshall, J. Tataryn, and F. Pollari. 2015. Estimates of foodborne illness-related hospitalizations and deaths in Canada for 30 specified pathogens and unspecified agents. *Foodborne Pathog. Dis.*12:820-7.

132. Thomas M. K., R. Murray, L. Flockhart, K. Pintar, F. Pollari, A. Fazil, A. Nesbitt, and B. Marshall. 2013. Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. *Foodborne Pathog. Dis.* 10:639-48.

133. Todd E. C. 1989. Costs of acute bacterial foodborne disease in Canada and the United States. *Int. J. Food Microbiol.* 9:313-26.

134. Tompkin R. B. 2002. Control of Listeria monocytogenes in the food-processing environment. *J.Food Prot.* 65:709-25.

135. Tompkin R.B. 2004. Environmental sampling - A tool to verify the effectiveness of preventative hygiene measures. p. 45-51. *In*, Presented at the 36th Symposium of the Swiss Society of Food Hygiene, vol. 95. Mitt. Lebensm. Hyg, Zurich.

136. U.S. Centers for Dsiease Ccontrol and Prevention. 2011. Estimates of Foodborne Illness in the United States. Available at: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed 17 April 2016.

137. U.S. Centers for Dsiease Ccontrol and Prevention. 2014. Surveillance for Foodborne Disease Outbreaks United States, 2012 : Annual Report. Available at: http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2012-508c.pdf. Accessed 17 April 2016.

138. U.S. Centers for Dsiease Ccontrol and Prevention. 2015. The Food Production Chain - How Food Gets Contaminated. Available at: http://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/production-chain.html. Accessed 21 July, 2016.

139. U.S. Food and Drug Administration. 2001. Bacteriological Analytical Manual.Rapid Methods for Detecting Foodborne Pathogens. Available at:

https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109652.htm. Accessed 8 April 2016.

140. U.S. Food and Drug Administration. 2009. FDA Food Code 2 Available at: http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm187947.htm#parta5-2. Accessed 13 July 2016.

141. U.S. Food and Drug Administration. 2009. FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types. Available at: http://www.fda.gov/downloads/Food/FoodSafety/RetailFoodProtection/FoodborneIllnessandRiskFactorReduction/RetailFoodRiskFactorStudies/UCM224682.pdf. Accessed 5 May, 2016.

142. U.S. Food and Drug Administration. 2013. Food Code. Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM374510.pdf. Accessed 15 July, 2016.

143. U.S. Food and Drug Administration. 2014. Evaluation and Definition of Potentially Hazardous Foods. Available at: http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094143.htm. Accessed 21 July 2016.

144. U.S. Food and Drug Administration. 2015. Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds Available at: https://www.fda.gov/downloads/scienceresearch/fieldscience/ucm298730.pdf. Accessed 21 May, 2016.

145. U.S. Food and Drug Administration. 2017. Draft Guidance for Industry: Control of Listeria monocytogenes in Ready-To-Eat Foods. Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM535981.pdf#page=35. Accessed 13 January 2017.

146. U.S. Food and Drug Adminsitraion. 2006. Managing Food Safety: A Regulator's Manual For Applying HACCP Principles to Riskbased Retail and Food Service Inspections and Evaluating Voluntary Food Safety Management Systems. Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM078159.pdf. Accessed 15 June 2016.

147. U.S.Department of Agriculture. 2002. Guidance for Beef Grinders and Suppliers of Boneless Beef and Trim Products Available at: http://www.haccpalliance.org/sub/food-safety/BeefGrindGuide.pdf. Accessed 26 April 2016.

148. U.S.Department of Agriculture. 2012. Listeria Control Program: Testing for Lm or an Indicator Organism. Available at: https://www.fsis.usda.gov/wps/wcm/connect/259fcaf4-ce2b-4ba1-8140-da873243040f/Chapter_3_Controlling_LM_RTE_guideline_0912.pdf?MOD=AJPERES. Accessed 13 January 2017.

149. U.S.Department of Agriculture. 2014. Cost Estimates of Foodborne Illnesses. Available at: http://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses. Accessed 13 July 2016.

150. Vallee J. C., and S. Charlebois. 2015. Benchmarking Global Food Safety Performances: The Era of Risk Intelligence. *J.Food Prot..* 78:1896-913.

151. World Health Organization. 2007. Consultation to Develop a Strategy to Estimate the Global Burden of Foodborne Diseases. Available at: http://apps.who.int/iris/bitstream/10665/43635/1/9789241595292_eng.pdf?ua=1. Accessed 16 March, 2016.

152. World Health Organization. 2009. Risk Characterization of Microbiological Hazards in Food. Available at: http://www.who.int/foodsafety/publications/micro/MRA17.pdf. Accessed 11 January, 2017.

153. World Health Organization. 2013. Salmonella (non-typhoidal). Available at: http://www.who.int/mediacentre/factsheets/fs139/en/. Accessed 23 June 2016.

154. World Health Organization. 2015. The Evolving World and Food safety. Available at: http://www.who.int/mediacentre/factsheets/fs399/en/. Accessed 21 July 2016.

155. World Health Organization. 2016. Foodborne diseases. Available at: http://www.who.int/topics/foodborne_diseases/en/. Accessed 13 April, 2016.

156. World Helaht Organization. 2013. Salmonella (non-typhoidal). Available at: http://www.who.int/mediacentre/factsheets/fs139/en/. Accessed 3 April 2016.

157. World Organization for Animal Health. 2015. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.09.09_Salmonellosis.pdf. Accessed 12 April, 2016.

ANNEXES

Annex1: The twenty-three control points used for the RBI method

Inspector Score Options			Master Points
Major	**Grave**		
		1	**Material**
10		1.1	Ambient Temperature
	50	1.2	Internal temperature of the food
10	50	1.3	Safety
10	50	1.4	Origin
10	50	1.5	Labeling Human health appearance
10	50	1.6	Lot identification
10	50	2	Method
10	50	2.1	Causes of survival, microbiological and unsafe utilization multiplication of food chemicals
10	50	2.2	Causes of introduction of pathogenic microorganisms or toxic or hazardous contaminants
10	50	2.3	Defrosting
10	50	2.4	Cooling / heating
10	50	2.6	Cleaning / Sanitation
10	50	2.7	Checks and documentation required
10	50	3	Man power
10	50	3.1	Hand washing and behavior of people in food establishment
10	50	3.2	Presence of adequate hand washing facilities
10	50	3.3	Dressing
10	50	3.4	State of apparent injuries and health
10	50	3.5	Movements of people in food establishment
10	50	3.6	Qualifications
10	50	4	Equipment
10	50	4.1	Cleanliness
10	50	4.2	State / nature, design / use and operation
10	50	5	Environment
10	50	5.1	Presence of Animals, insects or excreta
10	50	5.2	Environmental Sources of contamination (chemical, physical and microbiological)
10	50	5.3	Water supply: hot and cold

Annex 2: Types and percentage of positive samples for *L. monocytogenes* samples from FCS AND NFC surfaces in non-meat related RTE establishments

Establishment ID	No. of Samples	Positive *L. monocytogenes* samples	
		No. (%) of positive samples	Sites with positive samples detected
1	5	0 (0)	-
2	5	1 (20	Bain-marie/ chocolate machine
3	5	1 (20)	Floor of the processing room
4	5	0 (0)	-
5	5	0 (0)	-
6	5	1 (20)	Metallic- conveyor
7	5	0 (0)	-
8	5	2 (40)	Metalic tray used to bake and fruit conveyor