BC Centre for Disease Control
An agency of the Provincial Health Services Authority

PROVINCIAL Health Services AUTHORITY

Occurrence and distribution of *Listeria* species in facilities producing ready-to-eat foods under provincial inspection authority in British Columbia

FINAL REPORT

Prepared by:

Jovana Kovacevic and the Environmental Health Services Division
B.C. Centre for Disease Control

December 2, 2010

This page intentionally left blank

ACKNOWLEDGEMENTS

Project Team Members

Jovana Kovacevic, Food Safety Specialist, Food Protection Services, Environmental Health Services Division (EHSD), British Columbia Centre for Disease Control (BCCDC)

Lorraine McIntyre, Food Safety Specialist, Food Protection Services, EHSD, BCCDC

Lynn Wilcott, Acting Program Director, Food Protection Services, EHSD, BCCDC

Sion Shyng, Food Safety Specialist, Food Protection Services, EHSD, BCCDC

Brian Radke, Public Health Veterinarian, Food Protection Services, EHSD, BCCDC

Sarah Henderson, EH Scientist, Environmental Health Services Division, BCCDC

Tom Kosatsky, Medical Director, Environmental Health Services Division, BCCDC

British Columbia Regional Health Authorities

Fraser Health Authority

Tim Shum, Regional Director of Public Health Protection

Environmental Health Officers

Interior Health Authority

Ken Christian, Regional Director of Public Health Protection

Environmental Health Officers

Northern Health Authority

Lucy Beck, Regional Director of Public Health Protection

Environmental Health Officers

Vancouver Coastal Health Authority

Nick Losito, Regional Director of Public Health Protection

Environmental Health Officers

Vancouver Island Health Authority

Ann Thomas, Regional Director of Public Health Protection

Environmental Health Officers

Provincial Health Services Authority (PHSA) Laboratories

Judith Isaac-Renton, Public Health Laboratory Director, BCCDC Public Health Microbiology and Reference Laboratory, PHSA Laboratories

Environmental Microbiology

- Joe Fung, Section Head, Environmental Microbiology
- Julie Wong, Supervisor, Food Poisoning Laboratory, Environmental Microbiology
- Belinda Wong, Supervisor, Water Bacteriology Laboratory, Environmental Microbiology

General Bacteriology

- Linda Hoang, Program Head, Bacteriology and Mycology
- Ana Paccagnella, Section Head, Bacteriology and Mycology
- Laboratory technologists

Technical Support Department

- John Chan, Section Head
- Gin Lee, Technical Support

We are grateful for the cooperation of the 55 BC food processing facilities that participated in this survey.

The assistance of Sunny Mak, Senior Medical Geographer (Epidemiology Services, BCCDC) is much appreciated.

Thanks also to colleagues at the five BC Regional Health Authorities, the BC Ministry of Health Services, and the BC Ministry of Agriculture and Lands, who reviewed draft versions of this report.

EXECUTIVE SUMMARY

The contamination of food processing facilities by *Listeria monocytogenes*, the causative agent of human listeriosis, threatens the health of those who consume their products. *Listeria monocytogenes*, a bacterium found commonly in the environment, tolerates cold, moist, and salty conditions, facilitating its spread through facilities which process dairy, fish and meat products. The recent Canada-wide listeriosis outbreak associated with ready-to-eat (RTE) meats has reinforced the importance of monitoring and controlling *L. monocytogenes* through the chain of food production. Because RTE foods are commonly eaten without consumer preparation, *L. monocytogenes* can move to consumers from the facilities through the foods they produce. In British Columbia (BC), sampling for *L. monocytogenes* in food and food processing facilities is not required of producers who do not hold federal registration.

To date, there has been no comprehensive assessment of the prevalence of generic *Listeria,* the broad class of organisms which is often used as an indicator for the presence of *L. monocytogenes,* or of *L. monocytogenes* itself, in the food production sector in BC. To fill that gap, a 2009 survey was conducted in BC in order to estimate the prevalence of generic *Listeria* and *L. monocytogenes* in the foods and production environments of dairy, fish and meat facilities producing RTE foods under provincial inspection authority. An additional goal was to examine three production line sub-environments (non-food contact, close-to-food contact and food contact surfaces) in the facilities and to relate the prevalence of generic *Listeria* and *L. monocytogenes* in these sub-environments to that in foods produced in the facilities. The survey was initiated and conducted by the Food Protection Services section of the Environmental Health Services Division of the BC Centre for Disease Control (BCCDC). Collaborating with BCCDC were the Provincial Health Services Authority Laboratories and the five BC Regional Health Authorities.

From August to October 2009, 262 RTE food samples and 305 environmental swabs were collected from 53 BC dairy, fish, and meat RTE food production facilities. All dairies, all slaughterhouses, almost all fish facilities, and a sample of butchers and delis producing RTE foods under provincial inspection authority were included. Environmental swabs and food samples collected in facilities were analyzed using standard culture methods (MFHPB-30 and MFLP-74). Counts of *Listeria* colonies present in foods were performed followed by bacterial culturing in enrichment media. *Listeria monocytogenes* was differentiated from other species of *Listeria* using biochemical media.

Considering dairy, fish, and meat processors together, 9% of the foods tested harbored generic *Listeria (*all *Listeria* species together) and *L. monocytogenes* was isolated from 5% of the food products. RTE foods contaminated with *L. monocytogenes* were identified in the products of 5 of 12 fish processors surveyed, while the pathogen was found in none of the products collected from 17 dairy and 14 meat processors. Generic *Listeria* was found in 13%, and *L. monocytogenes* in 7% of the 305 environmental swab samples tested; in analysis by facility category, fish processing facilities (38%) showed the highest rates of contamination with *L. monocytogenes*. However, while *L. monocytogenes* was found in the processing environment of all three categories of production facilities, only in the fish processing facilities was it identified from food contact surfaces such as slicers, work tables, and cutting surfaces.

The survey results suggest that current practices for the control of *L. monocytogenes* in BC inspected dairy and meat facilities are effective in limiting food contamination with

L. monocytogenes. However, there is a lack of control of *L. monocytogenes* in RTE fish processing facilities under provincial inspection authority.

Based on our findings, BCCDC recommends:

- Reminding vulnerable populations in BC of the risk associated with the consumption of food products such as soft cheeses, deli-meats and smoked fish. This advice should be set in the context of the relatively low level of reported listeriosis in BC (a median of 11 cases per year from 2000-2009), despite the high level of morbidity and mortality associated with those severe listeriosis infections which do occur. In particular, until levels of *L. monocytogenes* in BC product drop, or until a province wide testing and labeling program can be put in place, pregnant women, immunocompromised individuals and the elderly should be advised of the risks associated with the high prevalence of *L. monocytogenes* in ready-to-eat smoked fish products.
- The development of evidence-based sampling guidelines for industry and government for effective monitoring of *Listeria* spp. and *L. monocytogenes* (and other foodborne pathogens) in RTE food processing facilities and their products to include: facility environments and products to sample, sampling procedures, frequency of sampling, and recommended follow-up actions.
- Enhanced training for food inspectors on the identification and control of *Listeria* spp. in processing environments.
- It is clear from the results of this study that BC fish processing facilities producing ready-to-eat foods require special attention. The purpose of further study and of enhanced inspection should be to improve food safety for consumers of RTE fish products. Specific recommendations for fish processing facilities include:
    - Identification of all provincially licensed fish processing facilities currently producing RTE foods in order to better track output and performance.
    - An assessment of Hazard Analysis Critical Control Points (HACCP) and other control measures in place in fish processing facilities, in conjunction with ongoing microbial testing of both the processing environment and foods to allow objective assessment of the effectiveness of means to audit and track facility hygiene.
    - Encouragement of research into how *Listeria* enters and spreads through the processing environment of smaller RTE food producers.
    - In the case of facilities where *Listeria* is identified, documentation of control measures where implemented, and their impact on the presence of *Listeria* in the processing environment and in fish products, to inform the optimal incorporation of practice-based learning into policies and procedures.
    - Establishment of a working group of stakeholders including industry, BC Ministry of Agriculture and Lands, BC Ministry of Health Services, BC Regional Health Authorities and BCCDC to consider the results of this and subsequent surveys of fish processing facilities, seek out system improvements and outline future policy directions.

- Additional sampling for *Listeria* spp. in BC inspected butcher and deli establishments which produce RTE meats, and continuing vigilance by both operators and food safety inspectors for breeches of hygiene which suggest the possibility of contamination by *Listeria* spp. at any BC inspected RTE processor.

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BAP	Defibrinated 5% sheep Blood Agar
BCCDC	British Columbia Centre for Disease Control
BCMAL	British Columbia Ministry of Agriculture and Lands
CFUs	Colony forming units
EHO	Environmental Health Officer
HBA	Horse Blood agar
LEB	*Listeria* enrichment broth
MFB	Modified Fraser broth
MID	Microgen® *Listeria* ID
Ox	Oxford agar
PAL	PALCAM agar
PHSA	Provincial Health Services Authority
RHA	Regional Health Authority
TSA	Trypticase soy agar

1. INTRODUCTION

1.1 *Listeria monocytogenes* and listeriosis

Despite the efforts of industry and food safety authorities to prevent microbiological contamination of food, pathogenic microorganisms continue to enter the food supply and cause both illness and economic damage. Among food-borne pathogens, *Listeria monocytogenes*, the organism responsible for a 2008 outbreak that led to the deaths of 23 Canadian consumers of ready-to-eat (RTE) meat products, is of particular concern *(71)*.

The *Listeria* genus is comprised of eight species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, *L. grayi* and recently discovered two novel species *L. marthii* and *L. rocourtiae (31,46,62)*. *Listeria monocytogenes* is primarily a human pathogen, causing a variety of infections in healthy and immuno-compromised individuals. In healthy people, the illness is manifested as febrile gastroenteritis or less frequently as cutaneous listeriosis *(80)*. Symptoms are generally non-specific and self-limited, including fever, diarrhea, headache and muscle pain; as a result, this type of listeriosis is believed to be under-diagnosed and rarely notified to public health authorities *(80)*. The most severe form of the disease, known as invasive listeriosis, is seen in individuals with impaired immune systems, pregnant women, newborns, the very young and the elderly *(18,31)*. In invasive listeriosis, symptoms are far more serious, and include meningitis, pneumonia, septicemia, spontaneous abortion, stillbirth and death *(18,31)*. Case-fatality rates associated with severe listeriosis have been reported in the order of 20 to 41% *(6,18,31,78,85)*. In the US, listeriosis is the cause of approximately 500 deaths per year or about 28% of all deaths associated with foodborne pathogens *(17)*.

Listeria monocytogenes also causes disease in animals, notably in domestic animals such as sheep, cattle, goats and birds *(76)*. Symptoms in livestock include encephalitis, septicemia and last trimester fetal loss *(76)*. *Listeria monocytogenes* has also been detected in fish and shellfish, although it does not appear to cause disease in these species. The contamination of fish and shellfish with *L. monocytogenes* is believed to derive from environmental sources and agricultural run-off *(76)*.

Of the other seven *Listeria* species, *L. ivanovii* is a known pathogen in animals, and is only rarely related to infections in humans *(76)*. Other species of *Listeria* are generally considered avirulent, with the exception of *L. seeligeri*, which has been reported to carry pathogenic traits *(44,84)* and in rare occasions has caused human listeriosis *(72)*.

In British Columbia, human listeriosis (i.e. invasive listeriosis) was made a disease reportable to public health authorities in 2002 following two large foodborne listeriosis outbreaks associated with consumption of BC-produced soft, mould-ripened, pasteurized milk cheese contaminated with *L. monocytogenes (5)*. In BC, from 6 to 22 (median 11) invasive cases of listeriosis have been reported each year during the period 2000-2009 *(8)*. In 2009, the year the survey reported here was conducted, 14 cases of listeriosis were reported in BC *(8)*.

While an average of 110 cases of listeriosis, few of them linked to any particular exposure, was reported in Canada during 2003-2007 *(85)*, a sharp increase in medical testing and reported cases of listeriosis occurred in 2008 in Canada (239 cases) and in BC (23 cases)

(5). The increase was largely associated with a nationwide outbreak linked to RTE processed meats.

Beyond Canada, other industrialized countries, notably the United States *(7)*, and France *(45)*, but not Germany *(59)* or Spain *(40)*, have reported a decreasing incidence of listeriosis during the last decade. The reported decrease of listeriosis in the US and France has been observed concurrent to the implementation of HACCP (Hazard Analysis Critical Control Points) programs in food processing facilities and more aggressive control measures for *L. monocytogenes* with zero or low tolerance for *L. monocytogenes* in RTE foods that support the growth of the organism *(80)*.

1.2 The presence and control of *L. monocytogenes* in food processing environments

Even with the implementation of HACCP and other food safety practices, the control of *L. monocytogenes* in food processing environments remains a challenge, as these bacteria are able to survive in very low temperatures, including refrigeration temperatures (i.e. < 4°C), high salt concentrations (i.e. 20%), high and limited oxygen environments, moderate to low acidity (i.e. pH 4 to 9.6)*(18,31)*, and due to their ability to attach to surfaces and form microbial communities known as biofilms *(67)*. With these traits and their wide distribution in the environment, *L. monocytogenes* have become common inhabitants of food processing establishments, with almost no food category free from their presence *(31)*. In particular, the presence of nutrients and wet surfaces in food processing environments creates favorable growth conditions for *L. monocytogenes* which, if neglected, can lead to biofilm formation and ongoing contamination of processing facilities *(76)*.

As different species of *Listeria* are often found living together *(67)*, the presence of any *Listeria* species in a food processing environment is an indication that conditions are favorable for the survival and potential growth of pathogenic *L. monocytogenes* *(66,76)*.

The sources and pathways of contamination by *L. monocytogenes* in food processing facilities appear to vary by facility type *(70)*. In facilities where foods undergo multiple handling steps, contaminated equipment and processing environments *(16)*, and less than meticulous food handler practices *(23)* have been reported to play a role in product contamination. It has been demonstrated that the complex equipment found in larger food processing facilities is difficult to clean and sanitize, and that these areas may harbor *L. monocytogenes* resulting in ongoing contamination of food products *(22,55)*. In smaller facilities, food product trays, crates, slicers, knives, carts and countertops may harbor *L. monocytogenes* leading to food contamination *(10,51,79)*. In most cases, however, it is difficult to determine the primary source of *L. monocytogenes* found in food processing environments *(50)*. In fact, considering the ubiquitous nature of *L. monocytogenes* and its wide dispersion in the environment, it is now recognized that the complete elimination of *L. monocytogenes* from processing environments and from the food production chain is difficult to achieve *(32,85)*. However, careful assessment of known hazards, combined with periodic microbial testing of foods and processing environments, worker training and ongoing vigilance are believed to be key to the reduction of contamination by *L. monocytogenes* to low levels *(34)*.

In her investigation of the Canada-wide listeriosis outbreak connected to processed meats, Weatherill *(85)* stressed the importance of validating sanitation plan effectiveness and monitoring environmental test results for *Listeria* over time to identify ongoing hazards. Testing of food products and food processing environments, including trend analysis of microbial results, was recognized as an important tool in the assessment of the safety of food products within a food processing facility *(85)*.

As a result of the federal investigation into the 2008 deli-meat outbreak *(85)*, recent amendments to the Canadian Food Inspection Agency's (CFIA) Meat Hygiene Manual of Procedures *(14)* have placed more attention at the federal level on testing for generic *Listeria* and/or *L. monocytogenes* in the manufacturing environment and in foods produced. More rigorous monitoring and increased frequency of inspection and sampling have been introduced at both government and processor levels in federally registered RTE meat processing facilities *(14)*. Additionally, Health Canada's revised policy on *L. monocytogenes* in all RTE foods *(34,35)* has the goal of increasing the effectiveness of preventive strategies and inspection procedures for the detection of generic *Listeria*, and especially *L. monocytogenes* in foods. The amendments to the previous policy include risk-based end-product (i.e. food) compliance criteria, trend analysis, and more detailed environmental and end-product sampling and testing guidelines *(33,34,35)*.

In BC, there are no provincial regulations or guidelines which refer specifically to generic *Listeria* and/or *L. monocytogenes* in the environment or in products from provincially licensed but not federally registered dairy, fish and meat processing facilities that produce RTE foods. While such facilities are routinely inspected, environmental and food product testing for generic *Listeria* or *L. monocytogenes* is not a requirement.

In dairy processing facilities regulated by the BC Milk Industry Act *(2)*, microbial testing of RTE dairy products for indicator microorganisms and/or pathogens (including *L. monocytogenes*) is required on at least six occasions during each six month period. In contrast, in fish processing facilities regulated by the BC Fish Inspection Act (Chapter 148; 1996) *(1)* there is no specific requirement for foods or environmental samples to be tested, although an inspector may collect samples during investigations or inspections. In meat processing facilities regulated by the Meat Inspection Regulation of the BC Food Safety Act (2004) *(4)* (slaughterhouses that produce RTE foods under provincial inspection authority) and meat facilities regulated by the Food Premises Regulation of the BC Public Health Act *(3)* (e.g. deli, butcher and other processors that produce RTE meat under provincial inspection authority), there are currently no specific regulations or guidelines for control of generic *Listeria* or *L. monocytogenes*; however, according to the BC Food Safety Act (Chapter 28) *(9)* inspectors may collect and examine any samples they deem appropriate.

1.3 Rationale for and partners in the current survey

In response to the 2008 Canada-wide listeriosis outbreak related to processed meats, two 2002 outbreaks linked to BC cheese manufacturers, and to the heightened attention to RTE meat facilities inspected by the Canadian Food Inspection Agency, the Environmental Health Services Division of BCCDC proposed a survey of generic *Listeria* and *L. monocytogenes* in

RTE food processing facilities subject to provincial inspection. Partners in this project were Provincial Health Services Authority (PHSA) Laboratories and the five BC Regional Health Authorities (RHAs).

Prior to the survey reported here, there had been no comprehensive assessment of the prevalence of generic *Listeria* or *L. monocytogenes* in food processing facilities in British Columbia. The goals of the survey were:

1. To estimate the prevalence of *Listeria* species, including *L. monocytogenes*, in the production environments of facilities producing RTE foods under provincial inspection authority in British Columbia, and in their products.
2. To compare the prevalence of generic *Listeria*, and in particular of *L. monocytogenes*, among three categories of RTE production facilities (dairy, fish and meat).
3. To estimate the prevalence of generic *Listeria*, and in particular of *L. monocytogenes*, across three production line sub-environments (non-food contact surfaces, close-to-food contact surfaces and food contact surfaces) in RTE food processing facilities, and to relate the prevalence of generic *Listeria* (including *L. monocytogenes*) in these processing environments to the prevalence of *L. monocytogenes* in foods produced at the facilities.
4. To assess the predictive value of screening for generic *Listeria* in the processing environment (and separately in ready-to-eat products) as a means to identify facilities where RTE product is contaminated with *L. monocytogenes.*

It was expected that the information gathered during the survey would:

a. Provide direction for future initiatives involving monitoring, control and prevention of *L. monocytogenes* in ready-to-eat foods and food processing environments in BC, and
b. Aid government agencies responsible for food safety in the evolution of safety regulations relevant to *L. monocytogenes* in RTE foods.

2. METHODS

2.1 Selection of food processing facilities

The selection of facilities was guided by three principles: the inclusion of representative facilities and RTE products from three major producer classes, namely dairy, fish and meat; coverage of the geographical territories of BC's five RHAs; and practicability within a three month sampling period.

All 18 dairy facilities producing RTE foods under provincial inspection authority in BC were considered for sampling.

From the list of fish and seafood processing facilities licensed by the British Columbia Ministry of Agriculture and Lands (BCMAL), 17 fish processing facilities were identified as producing RTE foods under provincial inspection authority. Four facilities with inconvenient production timetables or which were geographically isolated were not visited.

There were two sub-categories of meat RTE producers: slaughterhouse facilities producing RTE meat under provincial inspection authority; and other RTE meat processors, primarily delis and butcher shops. At the time of the survey, there were seven licensed slaughterhouse facilities, of which two were not producing RTE foods. Regional Health Authorities were asked to select for sample collection a total of 18 other RTE processors, based on regional quotas.

2.2 Sampling plan

In most instances, unannounced visits were conducted to collect samples; however, smaller facilities where production is occasional were contacted prior to sampling to ensure that the facility was in operation on that day. Dairy samples were collected by the two BCCDC Food Safety Specialists who regularly inspect them on behalf of the province, and Environmental Health Officers (EHOs) from BC's five RHAs visited fish facilities and meat producers (both slaughterhouse facilities and other meat processors). Two types of samples were collected at a single visit: environmental swab samples and RTE food products present at the facility. A detailed protocol for swabbing and food sample collection was provided to the EHOs and BCCDC Food Safety Specialists (Appendix 1).

In each facility, six environmental swab samples were requested to be taken from three areas of interest: non-food contact surfaces, close-to-food contact surfaces and food contact surfaces. The rationale for surface selection was based on previously published reports which examined the presence of generic *Listeria* and *L. monocytogenes* in food processing facilities *(60,65,82)*. Swabs were collected during food processing, at least three hours after the facility began operations, in order to increase the likelihood of obtaining positive results for generic *Listeria (14,81).* Sterile pre-wetted sponge applicators (Qualicum Scientific Ltd., Nepean, ON) were used to swab 30 by 30 cm areas, five times vertically and five times horizontally. Sponges were then placed in sterile bags and refrigerated for no more than 48 hours.

EHOs and BCCDC Food Safety Specialists were asked to collect six RTE food samples (approximately 150 g per sample) either aseptically in sterile sample bags or as pre-packaged consumer-ready product, per facility. Foods sampled had been produced on the day of the visit, or, in the case of foods normally aged prior to shipment (e.g. aged cheeses and meats), were collected at the end-stage of production ready for shipment to retailers.

Food samples were kept on ice or refrigerated prior to shipping to the laboratory, and were analyzed within 48 h of sample collection.

Samples recovered from dairy processing facilities included milk and fluid dairy products, hard and soft cheeses, yogurt and ice-cream.

Fish and seafood products primarily included cooked, heat dried or hot smoked salmon products with various flavors (e.g. teriyaki, honey garlic, Cajun, candied), as well as cold smoked and lox salmon products, smoked sablefish, sardines and cooked prawns.

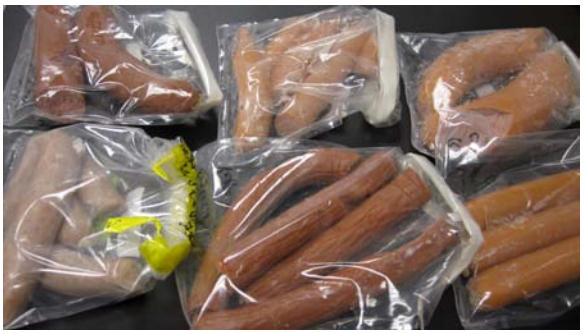
Dairy products in a dairy processing facility

Smoked salmon samples in whirl-pak™ bags

Table 1. List of surfaces that were requested for sampling in ready-to-eat dairy, fish and meat processing facilities for recovery of *Listeria* species.

Type of surface		
Non-food contact	**Close-to-food contact**	**Food contact**
Drain	Walls adjacent to food handling surfaces	Work-table
Sides/Legs :	Sides/Legs:	Packaging counter
Cart	Slicer	Food racks/shelves
Conveyor	Packaging table	Slicer
Vat	Shrink wrapper	Cutting board
Table	Work-table	Food bin
Refrigerator	Vacuum packer	Food display basket/bin/insert
Doors	Counter space	Food mold
Area under wash-sink	Silent cutter	Filler bowl
Support beams	Scale	Inside of vat pipes
Trolley wheels	Cup/jug filler	Cutting utensils
Bottom shelves of packaging/ wrapping tables	Show-case/display cooler door handle and interior	
Trolley wheels		

Meat samples tested included varieties of beef and pork sausages, “smokies”, pepperoni, prosciuttino salami, meatloaf, hot dogs/wieners, beef and deer jerky, turkey, chicken, ham and beef deli meats, as well as buffalo and bison salami and sausages.



RTE sausage samples in whirl-pak™ bags

Information describing the samples (food and environmental), was recorded on a single sample tracking form designed for the project (Appendix 2). It included: facility name and location, type of food processed/collected, date of collection, origin of the environmental samples and detailed description of the food samples including brand name and a best-before date or batch date.

2.3 Isolation of *Listeria* species from environmental swab samples

Environmental swab samples were analyzed for the presence of generic *Listeria* and *L. monocytogenes* according to Health Canada’s MFHPB-30 enrichment *(68)* method, with slight modifications (Figure 1).

Listeria Enrichment Broth (225 ml; LEB; Difco™, Becton, Dickinson and Co., Sparks, MD, USA) was added to bags containing sponges, after which they were incubated at 30°C for 24 h. Following gentle manual squeezing of the bags, a 0.1 ml aliquot of LEB culture was transferred to 10 ml of Modified Fraser Broth (MFB; formulation as per Health Canada’s MFHPB-30 method *(68)*) and incubated at 35°C for 48 h. In addition, after 24 h of incubation LEB samples were streaked onto PALCAM (PAL; Oxoid, Fisher Scientific Limited, Nepean, ON, Canada) and Oxford (Ox; Oxoid) selective agars for generic *Listeria*, and incubated at 35°C for 24 h and 48 h. Following the incubation, plates were examined for typical *Listeria* colonies at 24 h and 48 h, while MFB was examined for change in color. Additionally, MFB samples were streaked after 48 h of incubation onto selective plates (Ox and PAL); plates were examined for typical *Listeria* colonies after 24 and 48 h incubation at 35°C.

2.4 Isolation of *Listeria* species from food samples

Food samples were analyzed for the presence of *Listeria* spp. according to Health Canada’s MFLP-74 enumeration *(69)* and MFHPB-30 enrichment *(68)* methods, with slight modifications. Similar to environmental swab samples, 225 ml of LEB was added to 25 ml or 25 g of randomly selected analytical units of the food sample. Samples were then stomached for 30 seconds (Stomacher® 400, Seward Medical, Worthing, UK) after which 0.1 ml was spread plated onto duplicate plates of each PAL and Ox selective agars for *Listeria* spp. The plates were incubated at 35°C for 24 h and 48 h and at each time interval they were examined for typical *Listeria* colonies and the colony forming units (CFU) were counted.

LEB cultures were also incubated at 30°C for 24 h, after which time they were streaked onto PAL and Ox agars and 0.1 ml was transferred to 10 ml of MFB. Selective plates were incubated at 35°C for 24 h and 48 h, while MFB was incubated at 35°C for 48 h.

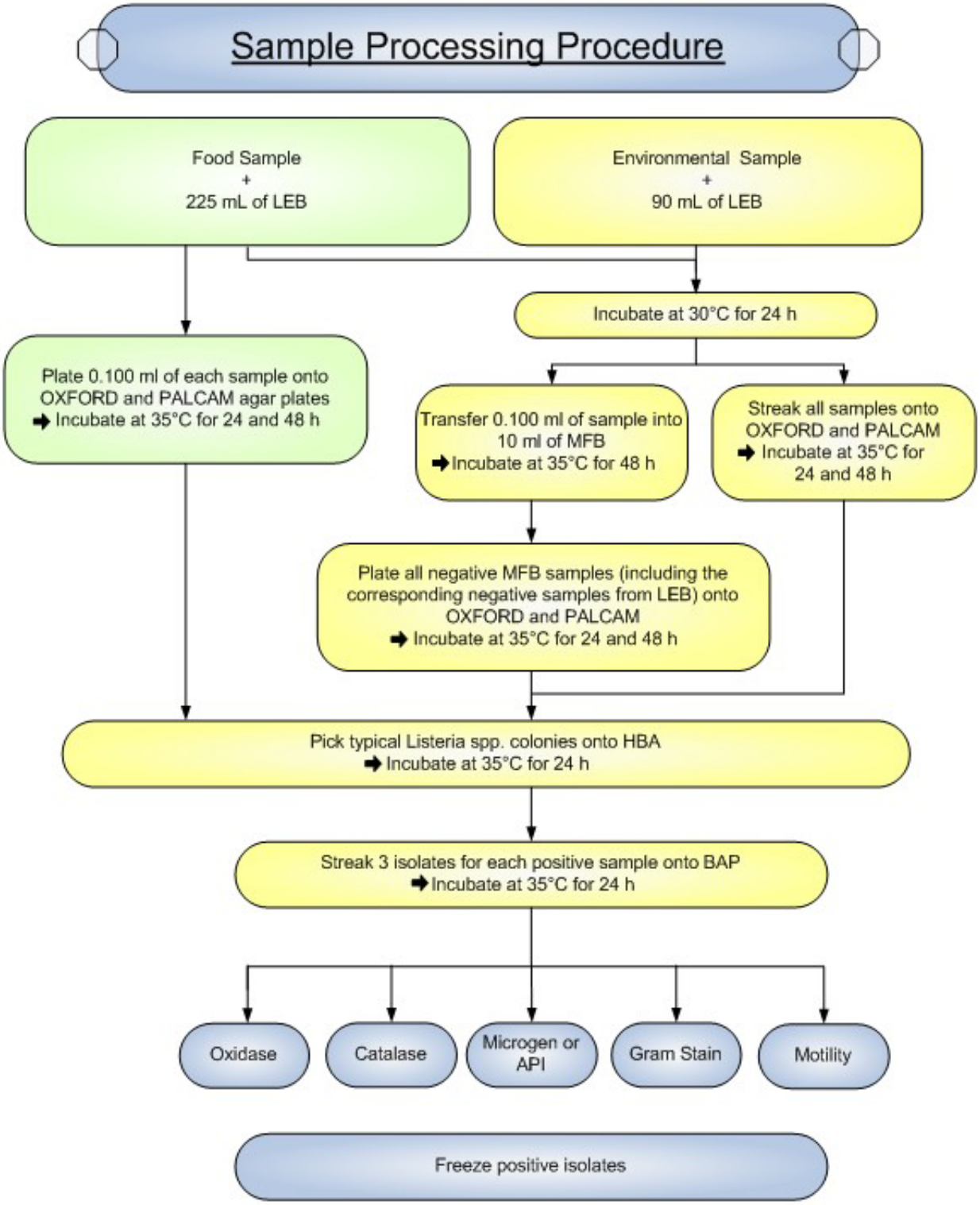


Figure 1. Modified processing procedure for recovery of *Listeria* species in food and environmental swab samples obtained from food processing facilities, based on Health Canada's MFHPB-30 and MFLP-74 culture methods.

Examination and/or streaking of the selective plates and MFB were performed as for the environmental samples, described above.

2.5 Isolate screening and confirmation

Screening of presumptive generic *Listeria* colonies was conducted using Horse Blood Agar (HBA; Dalynn Biologicals, Calgary, Alberta) selecting both β-hemolytic and non-hemolytic isolates, with emphasis on β-hemolytic isolates, after which at least three isolates from each sample were streaked onto defibrinated sheep blood agar (BAP; Oxoid) and optionally onto Trypticase Soy Agar (TSA; Difco™) to obtain pure colonies.

Further confirmation was based on Gram stain, catalase and oxidase reactions, and motility (Deep Listeria Motility; Difco™) at room temperature. Biochemical test strips (Microgen® Listeria ID, Microgen Bioproducts Ltd., Camberland, Surrey, U.K.) were used to differentiate *Listeria* species.

2.6 Reporting of positive results

Food and environmental samples that tested positive for *L. monocytogenes* were traced back to the facility and reported to the RHA where they are located. A list of recommended corrective measures to be applied to facilities testing positive for *Listeria* spp. (also termed generic *Listeria*) and *L. monocytogenes* was created by BCCDC (Appendix 3); however, the implementation of control measures was at the discretion of each RHA.

2.7 Statistical analysis

Facilities were included in statistical analyses on the presence of *Listeria* (both generic *Listeria* and *L. monocytogenes*, analyzed separately) in processing environments where at least one swab was collected from each of three environmental surface types: non-food contact, close-to-food contact and food contact. Facilities were included in statistical analyses on the presence of *Listeria* in food where at least four food samples were collected. For the association between *Listeria* in the processing environment and *Listeria* in food samples, only those facilities which met both sets of criteria were considered.

Besides tests comparing proportions of dairy, fish and meat **facilities** having *Listeria* positive results, statistical tests comparing proportions of positive **samples** were also conducted. These tests of samples (as contrasted with tests of facilities) took into account the aggregation of positive results by facility. The same considerations were applied to the estimation of the proportion of food and environmental samples testing positive for *Listeria* within each processor category. This relatively conservative assumption would be expected to lead to wide confidence intervals around the prevalence estimates.

All analyses were performed using R software (version 2.10.1; R Foundation for Statistical Computing, Vienna, Austria). The statistical methods used to address different hypotheses are described below.

1. Two-tailed Fisher's exact test was used to assess differences in the proportions of facilities with environment swabs or food samples positive for generic *Listeria* and *L. monocytogenes*, analyzed separately, among the dairy, fish and meat categories, at a 5% level of significance.
2. The association of environmental swab (or separately, food sample) positivity with facility type was assessed by logistic regression.

These analyses adjusted for the effect of specific facilities by weighting the samples from individual facilities according to the probability of finding positive samples within that facility.

3. Contingency table analysis was used to assess the probability of finding *Listeria* (generic *Listeria* and *L. monocytogenes*, analyzed separately) in foods at a facility given that *Listeria* were found in the environment at that facility. In contingency tables, each facility was counted in one of four categories:
   (**A)** *Listeria* found in food and *Listeria* found in environment;
   (B) *Listeria* found in food and not found in environment;
   (C) *Listeria* not found in food and found in environment; and
   (D) *Listeria* not found in food and not found in environment.
   The odds of finding *Listeria* in foods where it is found in the environment were calculated as A / C, and the odds of finding *Listeria* in food where it is not found in the environment were calculated as B / D. The ratio of these odds [(A x D) / (B x C)] indicated the strength of the association between *Listeria* in the environment and *Listeria* in food.

3. RESULTS

3.1 Facilities sampled

Fifty-three (53*) RTE food processing facilities were visited between July and October 2009. These included 17 dairy, 13 fish, and 23 meat RTE processors. Facilities were visited in the territories of all five RHAs (Figure 2).

Of the 18 non-federally registered dairy facilities producing RTE product, all 17 in operation as of July 2009 were visited.

Of the approximately 17 non-federally registered RTE fish processing facilities, 13 facilities were visited.

The five provincially licensed and inspected slaughterhouses actively producing RTE meat products and 18 of the other meat processors selected by RHAs constituted the sample of meat processing facilities.

The number of BC facilities under provincial inspection authority, the number of facilities selected, those visited and sampled and those that met inclusion criteria for statistical analysis as a facility unit are presented in Table 2.

3.2 Sample results

Overall, 567 samples were collected (this number includes samples taken from facilities treated statistically as facility units as well as samples from facilities which were not included in statistical comparisons by facility unit): 262 ready-to-eat dairy, fish and meat products and 305 environmental swab samples.

Of the 305 environmental swabs taken, 101 were from non-food contact surfaces, 101 from close-to-food contact surfaces, and 103 from food contact surfaces.

Table 2. The number of facilities producing ready-to-eat foods under provincial inspection authority included in the survey.

Facility type	Facilities under provincial inspection authority	Facilities selected for the survey	Facilities sampled	Facilities sampled that met statistical inclusion criteria for environmental swabs*	Facilities sampled that met statistical inclusion criteria for food†
Dairy	18	18	17	17	17
Fish	17‡	17	13	13	12
Meat	> 100	28§	23**	21	14
Total	-	63	53	51	43

* 3 or more environmental swab samples collected per facility, of which at least one collected for each of the three sampling area categories: non-food contact, close-to-food contact and food contact surfaces.
† 4 or more food samples collected per facility.
‡ Represents approximate number of RTE facilities licensed at the time of the survey, and does not include processors licensed to produce both meat and fish RTE products.
§ 7 class A slaughterhouses and 21 other RTE meat processors.
** 5 class A slaughterhouses and 18 other RTE meat processors.

*In addition to the 53 dairy, fish and meat RTE processors visited, during the course of the project, two tofu processing facilities were visited. Overall, 24 samples were collected: 11 RTE food products and 13 environmental swabs. Food samples included tofu fried puffs, soft and medium firm tofu, dessert tofu, sweetened and unsweetened soy drinks, black soy milk and bean curd. Environmental swabs were taken of non-food contact surfaces such as: drains, benches, light switches and legs of the machinery, close-to-food surfaces such as: walls adjacent to food handling surfaces and sides of the coagulation machinery, as well as the food contact surfaces including filling tanks, trays, work- and packaging tables. None of the tested food or environmental samples were positive for generic *Listeria*, or *L. monocytogenes*.

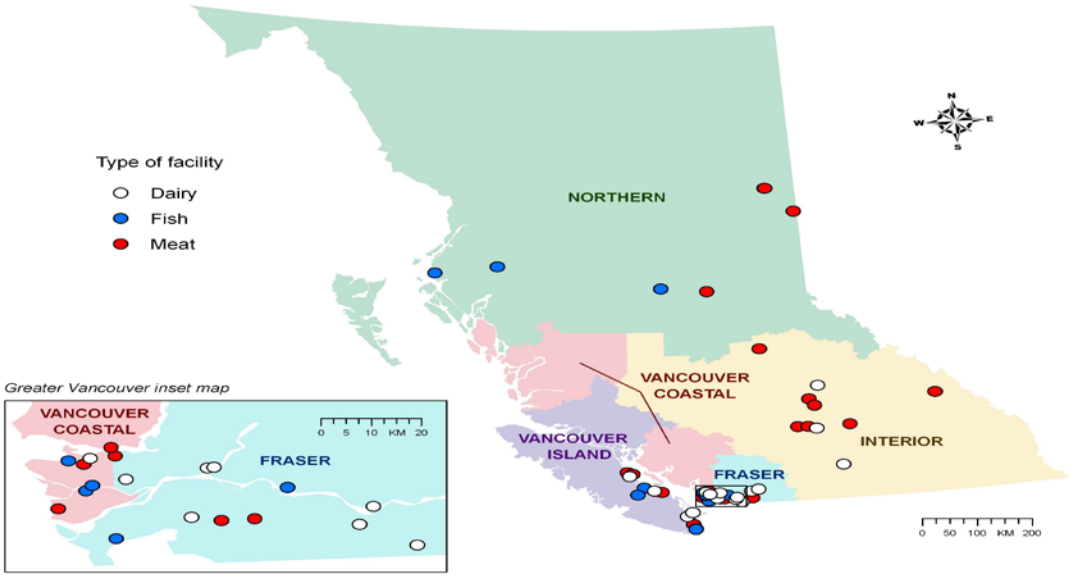


Figure 2. Geographic distribution of facilities producing ready-to-eat foods under provincial inspection authority (n=53) visited during the survey that assessed the prevalence of generic *Listeria* in food facilities, by Health Authority regions in British Columbia.

Listeria spp., referred to as generic *Listeria*, were recovered from 30% of non-food contact surface swabs, 5% of close-to-food contact surface swabs and 6% of food contact surface swabs (Table 3).

Table 3. Prevalence of generic *Listeria* and *L. monocytogenes* (*L.m.*) in environmental samples* from 51 RTE food processing facilities that met the sample number criteria.

	Surface Sampled						All surfaces	
	Non-food-contact		Close to food		Food contact			
Facility type	Positive for *Listeria* spp. (%)	**Positive for *L.m.* (%)**	Positive for *Listeria* spp. (%)	**Positive for *L.m.* (%)**	Positive for *Listeria* spp. (%)	**Positive for *L.m.* (%)**	Positive for *Listeria* spp. (%) (95% CI†)	**Positive for *L.m.* (%), (95% CI†)**
Dairy	24	**12**	3	**3**	0	**0**	9 (0.7-17)	**5 (0-12)**
Fish	50	**27**	15	**12**	23	**12**	29 (11-48)	**17 (2-32)**
Meat	22	**7**	0	**0**	0	**0**	7 (3-12)	**2 (0-7)**
ALL	**30**	**13**	**5**	**4**	**6**	**3**	**13 (8-19)**	**7 (2-11)**

*Prevalence odds ratios (OR) for the probability of finding generic *Listeria* and *L. monocytogenes*, analyzed separately, in the processing environments of the three RTE food producer categories adjusted by weighting the samples from individual facilities, according to the probability of finding positive swab samples within that facility:

For generic *Listeria*: **Fish vs. Dairy** (OR 2.99; Confidence Interval (CI: 0.99-8.96); p= 0.051); **Fish vs. Meat** (**OR 2.92**; CI (1.03-8.20); **p=0.042**); **Meat vs. Dairy** (OR 1.02; CI (0.33-3.16); p=0.97).

For *L. monocytogenes*: **Fish vs. Dairy** (OR 3.29; CI (0.83-13.12); p=0.091); **Fish vs. Meat** (OR 5.13; CI (1.15-22.82); **p=0.032**); **Meat vs. Dairy** (OR 1.56; CI (0.29-8.37); p=0.60).

†CI, confidence interval, calculated as the average of facility-specific proportions with the standard error of the mean being estimated by the standard deviation over the square root of the number of facilities.

Listeria monocytogenes was found in 13% of non-food contact surface swabs, 4% of close-to-food contact surface swabs and in 3% of food contact surface swabs (Table 3).

Of 51 facilities that met the criterion of at least one swab collected in each of the three sampling areas, generic *Listeria* was recovered from the processing environments of 21 (41%) of which *L. monocytogenes* was identified in 11 (22%). Non-food contact surfaces were contaminated with generic *Listeria* and *L. monocytogenes* in 21 (41%) and 10 (20%) facilities, respectively. Generic *Listeria* (in all cases also *L. monocytogenes*) were recovered from close-to-food contact surfaces in 4 (8%), while 5 (10%) and 2 (4%) facilities had generic *Listeria* and *L. monocytogenes*, respectively, recovered from food contact surfaces (Figure 3).

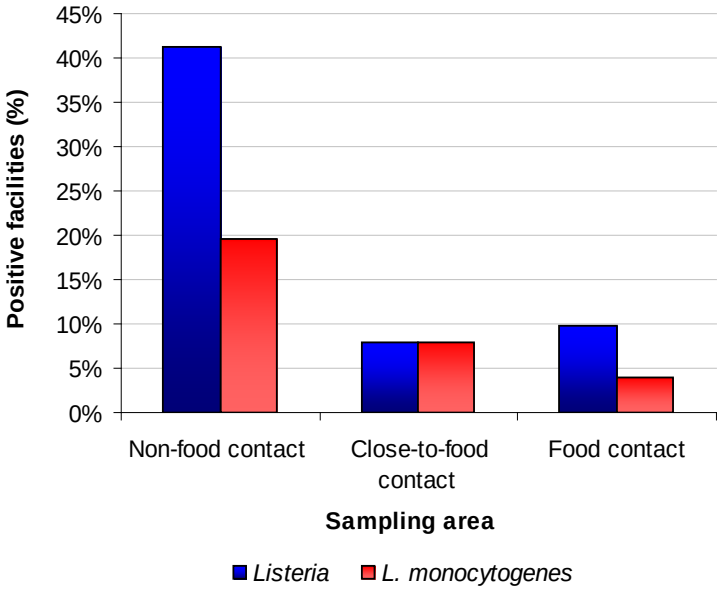


Figure 3. The proportion of facilities meeting the criterion of at least one swab collected in each of the three sampling areas, having environmental swab samples positive for generic *Listeria* and *L. monocytogenes* by sampling area.

There were differences (not statistically significant=NS) in the proportions of RTE dairy, fish, and meat facilities with at least one environmental swab sample positive for generic *Listeria* and *L. monocytogenes* (Figure 4).

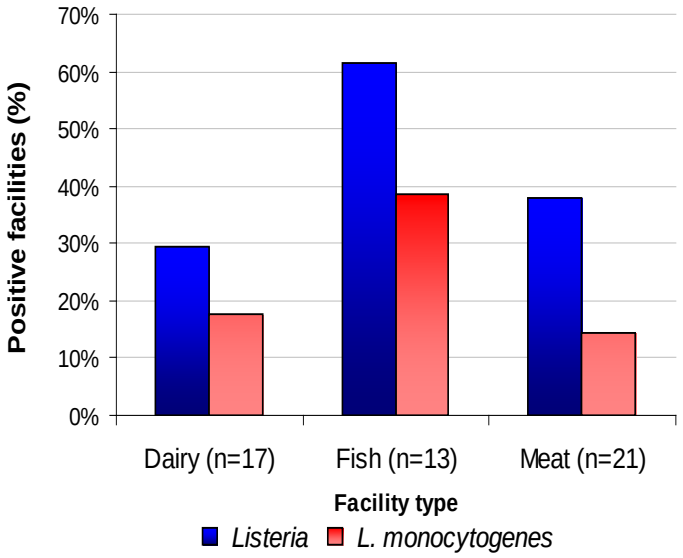


Figure 4. The proportion of facilities meeting the criterion of at least one swab collected in each of the three sampling areas, having environmental swab samples positive for generic *Listeria* and *L. monocytogenes* by facility type.

Considering the sub-environments of processing facilities, differences were small in the proportions of dairy, fish, and meat facilities having swabs of drains and other non- food contact surfaces positive for generic *Listeria* and *L. monocytogenes*. The same comparison for close-to food contact surfaces indicated a statistically higher proportion of fish facilities positive for generic *Listeria* and *L. monocytogenes* compared to meat facilities (both 3/13 *versus* 0/21, p=0.048), but not in comparisons between other facility categories.

Further, the proportion of fish facilities with one or more food contact surfaces positive for generic *Listeria* was significantly higher than the proportion of dairy (5/13 *versus* 0/17, p=0.009) and meat (5/13 *versus* 0/21, p=0.005) facilities. Among all three facility categories, only in RTE fish processors was *L. monocytogenes* recovered from food contact surfaces (0/17, 2/13 (fish), 0/21: NS).

Overall, 262 ready-to-eat food samples were collected. Generic *Listeria* were recovered from 9% of the samples, while *L. monocytogenes* was found in 5% of the tested food samples (Table 4).

Of 43 facilities visited which met the criterion of at least four RTE food samples collected in a facility, 8 (19%) and 5 (12%) had generic *Listeria* and *L. monocytogenes*, respectively, recovered from RTE food samples.

There was a higher proportion of fish facilities compared to dairy facilities where one or more food samples was positive for generic *Listeria* (6/12 *versus* 0/17, p=0.002) and *L. monocytogenes* (5/12 and 0/17, p=0.007). Proportionally more fish than meat facilities had a food positive for generic *Listeria* (6/12 *versus* 2/14, NS) and *L. monocytogenes* (5/12 *versus* 0/14, p=0.012) (Figure 5).

Table 4. Prevalence of generic *Listeria* and *L. monocytogenes* in ready-to-eat (RTE) food samples* from RTE food processing facilities where at least one RTE food sample was collected (n=50).

Facility type	Food samples positive (%) (95%CI†)	
	Generic *Listeria*	*L. monocytogenes*
Dairy (n=100)	0	**0**
Fish (n=71)	28 (5-50)	**20 (2-37)**
Meat (n=91)	3 (0-9)	**0**
Total (n=262)	**9 (2-14)**	**5 (0-9)**

*Prevalence odds ratios (OR), for the probability of finding generic *Listeria* and *L. monocytogenes*, analyzed separately, in foods of three categories adjusted by weighting the samples from individual facilities according to the probability of finding positive samples within that facility:

For generic *Listeria*: **Fish vs. Dairy** (OR Infinite; Confidence Interval (CI) non-calculable); **Fish vs. Meat** (OR 5.19; CI (1.06-25.43); p=0.042); **Meat vs. Dairy** (OR Infinite; CI non-calculable).

For *L. monocytogenes*: **Fish vs. Dairy** (OR Infinite; CI non-calculable); **Fish vs. Meat** (OR Infinite; CI non-calculable); **Meat vs. Dairy** (OR 1.0; CI non-calculable).

†CI, confidence interval, calculated as the average of facility-specific proportions with the standard error of the mean being estimated by the standard deviation over the square root of the number of facilities.

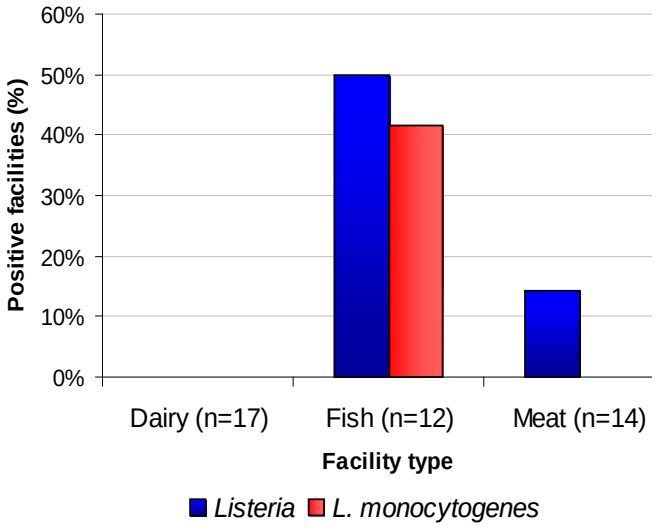


Figure 5. The proportion of facilities meeting the criterion of at least four ready-to-eat food samples collected, having food samples positive for *Listeria* and *L. monocytogenes*.

Of the 43 facilities that met the criteria of at least one swab collected in each of the three sampling areas and at least four RTE food samples collected, 11 (26%) had generic *Listeria* recovered only from the processing environment, 7 (16%) had *Listeria* recovered from both environment and RTE foods, and 1 (2%) had *Listeria* isolated only from foods.

Contingency table analysis revealed that facilities where one or more foods were contaminated with generic *Listeria* were 15 times (7/18 versus 1/25, prevalence odds ratio 15.3; p=0.005) more likely to have had generic *Listeria* found in swabs from the processing environment than facilities with no generic *Listeria* positive foods.

Further, facilities where one or more foods were contaminated with *L. monocytogenes* were extremely (5/18 versus 0/25, prevalence odds ratio infinite, p=incalculable) more likely to

have generic *Listeria* found in swabs from the processing environment than facilities with no *L. monocytogenes* positive foods. Interestingly, *L. monocytogenes* was never found in foods where generic *Listeria* was not present somewhere in the processing environment.

The joint presence of *L. monocytogenes* in foods and generic *Listeria* in swabs of the processing environment is shown in Figure 6. Each line of the figure represents a single facility. It illustrates the finding that non-food contact surfaces were contaminated with generic *Listeria* in facilities of all three categories, but that food-contact surfaces were contaminated only in RTE fish facilities. It also shows that *L. monocytogenes* was found only in RTE fish products. More importantly, it shows that in all facilities where product was contaminated with *L. monocytogenes*, generic *Listeria* was present in the processing environment.

3.3 Dairy processing facilities and their RTE food products

The 17 dairy processing facilities visited each had swabs taken from all three environmental sampling areas and had at least four food product samples collected. Generic *Listeria* were found in the production environments of 5 (29%) facilities, while *L. monocytogenes* was recovered from 3 (18%) facilities.

3.3.1 Generic *Listeria* and *L. monocytogenes* contamination of the dairy processing environment

Of 102 environmental swabs collected from dairy processing facilities, 9 (9%) tested positive for generic *Listeria*.

Eight of the nine positive surface swabs came from non-food contact surfaces, such as drains, area under wash sink, conveyor and the surfaces around them.

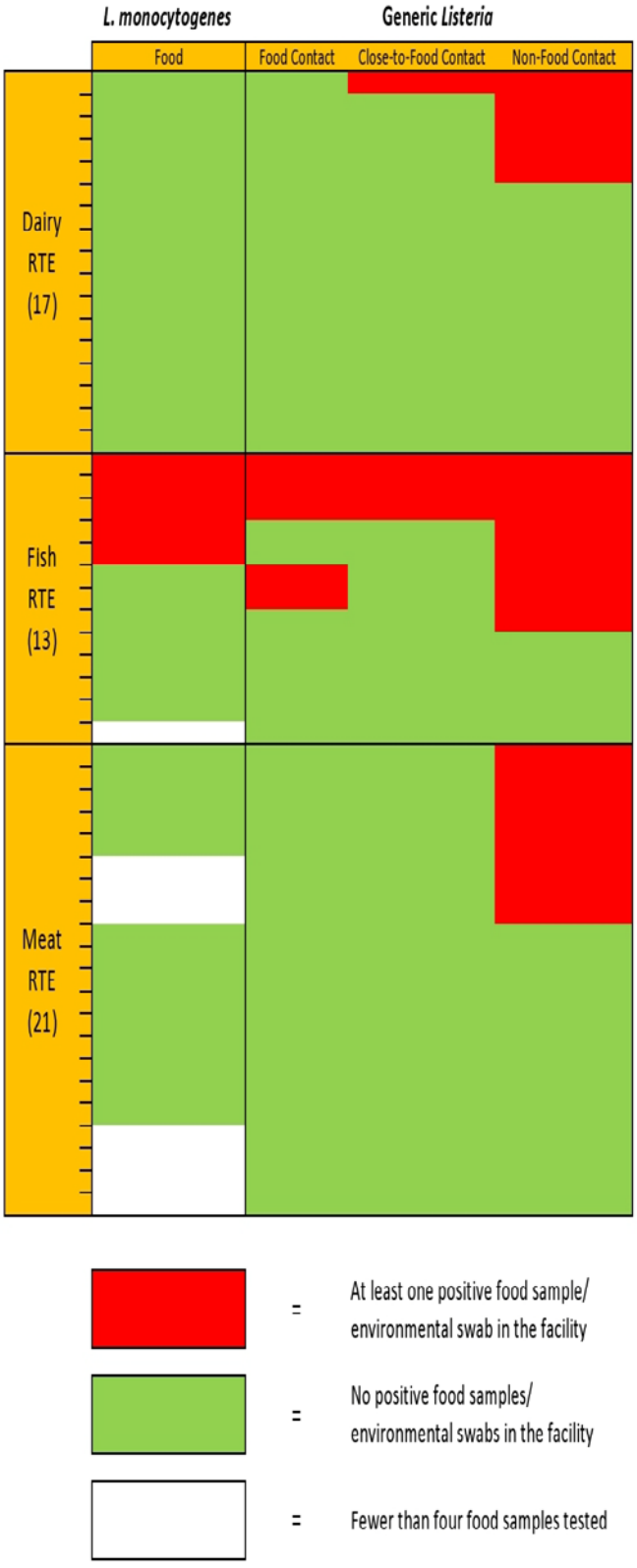


Figure 6. The joint presence of *L. monocytogenes* in food and generic *Listeria* in the processing environment, for facilities with at least one swab from all three sampling areas.

In one instance (1/9), a draining rack, considered a close-to-food contact surface, was also positive for generic *Listeria.* None of the swabs from food contact surfaces in dairy facilities tested positive for generic *Listeria.*

Listeria monocytogenes was recovered from three (3/8) of the generic *Listeria* positive non-food contact surface swabs (i.e. drains) and from one (1/1) positive close-to food contact surface swab (i.e. draining rack). Species of *Listeria* other than *L. monocytogenes* recovered from environmental swabs of dairy facilities included *L. innocua* and *L. seeligeri* (Table 5).

None of 100 RTE dairy food samples tested harbored generic *Listeria* (Table 6).

3.4 Fish processing facilities and their RTE food products

Of the 13 fish processing facilities visited, all had swabs taken from three environmental sampling areas and 12 facilities met the criterion of at least four RTE food samples collected. Processing environment swabs were positive for generic *Listeria* in 8 (62%) facilities, while *L. monocytogenes* was recovered from 5 (38%) facilities. Similarly foods were positive for generic *Listeria* in 6 (50%) facilities, while *L. monocytogenes* was found in foods from 5 (42%) facilities.

3.4.1 Generic *Listeria* and *L. monocytogenes* contamination of the fish processing environment

Of 78 environmental swabs collected from fish processing facilities, 23 (29%) tested positive for generic *Listeria*. The majority of surface swabs positive for generic *Listeria* were non-food contact surfaces (13/23), followed by food contact surfaces (6/23) and close-to-food contact surfaces (4/23) (Table 3).

Non-food contact surfaces contaminated with generic *Listeria* included drains, legs of a sink, tables and carts. Generic *Listeria* were also found on food contact surfaces, such as cutting boards, work-tables and shelves holding RTE products, as well as close-to-food surfaces, such as a wall behind slicers, slicer legs, a packaging table, and work-table shelving.

Table 5. Species of *Listeria* isolated from environmental samples by type of food processing facility.

	Environmental samples							
	Dairy (n[a]=102)		**Fish** (n=78)		**Meat** (n=125)		**Total** (n=305)	
Species	No. positive	%	No. positive	%	No. positive	**%**	No. positive	%
L. monocytogenes	**5**	**4.9**	**13**	**16.7**	**3**	**2.4**	**21**	**6.9**
Other species								
L. innocua	3	2.9	1	1.3	3	2.4	7	2.3
L. welshimeri	0	0.0	1	1.3	3	2.4	4	1.3
L. seeligeri	2	2.0	10	12.8	0	0.0	12	3.9
No. of samples positive for generic *Listeria* [b]	9	8.8	23	29.5	9	7.2	41	13.4

[a] "n" represents the number of environmental samples collected in each facility type.
[b] Numbers do not add up in each column as some samples were positive for more than one species of *Listeria.*

Table 6. Species of *Listeria* isolated from food samples (n=262) by type of food processing facility.

Species	Food samples							
	Dairy (n[a]=100)		**Fish** (n=71)		**Meat** (n=91)		**Total** (n=262)	
	No. positive	%	No. positive	%	No. positive	**%**	No. positive	%
L. monocytogenes	**0**	**0**	**14**	**20**	**0**	**0**	**14**	**5**
Other species								
L. innocua	0	0	8	11	1	1	9	3
L. welshimeri	0	0	2	3	2	2	4	2
No. of samples positive for generic *Listeria* [b]	0	0	20	28	3	3	23	9

[a] "n" represents the number of food samples collected in each facility type.
[b] Numbers do not add up in each column as some samples were positive for more than one species of *Listeria.*

Listeria monocytogenes was recovered from 13 of 23 contaminated environmental swabs (Table 3).

Seven of the *L. monocytogenes* positive swabs came from non-food-contact surfaces; three from close-to-food contact surfaces and three were taken from food contact surfaces. Contaminated surfaces included drains, legs of a sink, work-table, cart and packaging table, as well as a cutting board, slicer and a work-table in direct contact with RTE food. Species of *Listeria* other than *L. monocytogenes* recovered from environmental surfaces in fish processing facilities included *L. seeligeri*, *L. innocua* and *L. welshimeri* (Table 5).

The list of fish facilities with environmental samples contaminated with generic *Listeria* and *L. monocytogenes* is presented in Table 7. Three (38%) of the eight contaminated facilities had environmental surfaces in all three sampling areas contaminated with generic *Listeria*, 2 (25%) had both non-food contact and food contact surfaces contaminated with the bacteria, while 3 (38%) facilities had contamination only of non-food contact surfaces.

Overall, *L. monocytogenes* was found in processing environments in 5 of the 13 fish facilities visited. In one facility *L. monocytogenes* was found on all three types of surfaces sampled (non-food contact, close-to-food and food contact surfaces). A second facility had a close-to-food contact and two food contact surfaces contaminated with *L. monocytogenes*. A third *L. monocytogenes* contaminated facility had the bacterium recovered from both non-food contact and close-to-food contact surfaces. Two facilities had *L. monocytogenes* found on non-food contact surfaces only.

3.4.2 Generic *Listeria* and *L. monocytogenes* contamination of RTE fish products

Of 71 RTE fish samples analyzed, 57 (80%) were hot smoked, heat dried or cooked and 14 (20%) were cold smoked.

Generic *Listeria* were recovered from 20 (28%) fish samples (Table 8), of which the majority (17/20) were hot smoked, heat dried or cooked, followed by cold smoked products (3/20). The list of contaminated

foods is presented in Table 8. Bacterial counts in 14 of the contaminated products were less than 100 CFU/g (Table 9). In 6 of the 20 (30%) contaminated food products counts greater than 100 CFU/g were observed, with three hot smoked salmon products being grossly contaminated (i.e. greater than 30,000 CFU/g) (Table 9). Only 1 of the 3 contaminated cold smoked products had bacterial counts greater than 100 CFU/g (Table 9).

Listeria monocytogenes was recovered from 14 of the RTE fish samples including 13 hot smoked, heat dried or cooked products, and one cold smoked salmon product (Table 8). Further, *L. monocytogenes* was recovered from four of the contaminated products that had generic *Listeria* counts greater than 100 CFU/g; three of which were grossly contaminated with more than 30,000 CFU/g (Table 9).

Species of *Listeria* other than *L. monocytogenes* found in RTE fish products included *L. innocua* and *L. welshimeri*, (Table 6).

Of the six facilities with food samples contaminated with generic *Listeria*, four had several food samples positive, and in two facilities only one food sample was positive for generic *Listeria* (Table 8).

3.5 Meat processing facilities and their RTE food products

Of the 23 meat processing facilities visited, in 21 (5 slaughterhouses and 16 other meat processors) environmental swabs were taken in all three sampling areas, while 14 facilities (5 slaughterhouses and 9 other meat processors) had at least four food products collected.

Generic *Listeria* was found in the processing environment of 8 (38%) meat facilities, while *L. monocytogenes* was recovered from 3 (14%).

Only 2 of 14 (14%) RTE meat facilities, from which four or more food samples were taken, had generic *Listeria* found in foods, while none had *L. monocytogenes* recovered from foods.

3.5.1 Generic *Listeria* and *L. monocytogenes* contamination of the meat processing environment

Of 125 environmental swabs collected from processing environments of meat facilities, 30 were from slaughter facilities and 95 came from other meat processors (butcher and deli shops). Generic *Listeria* was recovered from 7% of the environmental swabs (Table 3). The majority of the contaminated swabs (6/9) came from deli and butcher shops, while three swabs (3/9) from slaughter meat facilities were positive for generic *Listeria*.

All nine of the positive environmental swabs came from non-food contact surfaces; eight were taken from drains and floors adjacent to drains, and one was a swab of a cart transporting raw meat into the RTE production area. None of the close-to-food contact (0/41) and food contact surfaces (0/43) tested positive for generic *Listeria*.

Listeria monocytogenes was cultured from three of the nine generic *Listeria* positive environmental swabs. All three were collected in deli and butcher shops and all were taken from drains. None of the swabs collected from slaughter facilities harbored *L. monocytogenes*. Species of *Listeria* other than *L. monocytogenes* found in the environmental swabs from slaughter facilities and deli and butcher shops included *L. innocua* and *L. welshimeri* (Table 5).

Table 7. Prevalence of generic *Listeria* and *L. monocytogenes* in the environment of 13 RTE fish processing facilities.

	Sample type							
	Non-food-contact surface [a]		Close to food surface [b]		Food contact surface [c]		All surfaces	
Facility No.	Positive for generic *Listeria* / Tested	**Positive for *L.m.* / Tested**	Positive for generic *Listeria* / Tested	**Positive for *L.m.* / Tested**	Positive for generic *Listeria* / Tested	**Positive for *L.m.* / Tested**	Positive for generic *Listeria* / Tested	**Positive for *L.m.* / Tested**
F19	2/2	**2/2**	2/2	**1/2**	1/2	**1/2**	5/6	**4/6**
F20	2/2	**2/2**	1/2	**1/2**	1/2	0/2	4/6	**3/6**
F21	2/2	**2/2**	0/2	0/2	0/2	0/2	2/6	**2/6**
F24	1/2	0/2	0/2	0/2	1/2	0/2	2/6	0/6
F25	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6
F26	1/2	0/2	0/2	0/2	1/2	0/2	2/6	0/6
F28	2/2	0/2	1/2	**1/2**	2/2	**2/2**	5/6	**3/6**
F29	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6
F30	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6
F31	1/2	**1/2**	0/2	0/2	0/2	0/2	1/6	**1/6**
F32	2/2	0/2	0/2	0/2	0/2	0/2	2/6	0/6
F33	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6
F18	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6
Total (%)	**13/26 (50)**	**7/26 (27)**	**4/26 (15)**	**3/26 (12)**	**6/26 (23)**	**3/26 (12)**	**23/78 (29)**	**13/78 (17)**

[a] Drains, floors, legs and sides of tables, underneath shelves, sides and wheels of carts.
[b] Walls adjacent to food handling areas, legs of food processing equipment.
[c] Work-tables, cutting boards, racks, slicers, fillers.

Table 8. Prevalence of generic *Listeria* and *L. monocytogenes* in foods in 13 RTE fish processing facilities.

	No. of samples positive/ No. of samples tested		Foods	
Facility No.	Generic *Listeria*	***L. monocytogenes***	Positive for generic *Listeria*	Positive for *L. monocytogenes*
Facilities in which at least 4 food samples were collected				
F19	1/4	**1/4**	Smoked salmon	Smoked salmon
F20	4/7	**4/7**	Salmon candy (3 samples), smoked sablefish	Salmon candy (3 samples), smoked sablefish
F21	4/6	**2/6**	Salmon leather (3 samples), cold smoked salmon	Salmon leather, cold smoked salmon
F24	0/6	0/6		
F25	0/6	0/6		
F26	0/6	0/6		
F28	4/6	**2/6**	Smoked salmon, salmon candy, lox whole salmon, lox sliced salmon	Smoked salmon, salmon candy
F29	1/6	0/6	Cooked prawns	
F30	0/6	0/6		
F31	6/6	**5/6**	Candied salmon, salmon jerky, shrimp meat, smoked salmon with different flavorings (3 samples)	Salmon jerky, shrimp meat, smoked salmon with different flavorings (3 samples)
F32	0/6	0/6		
F33	0/6	0/6		
Sub-total	**20/71**	**14/71**		
Facilities in which fewer than 4 food samples were collected				
F18	N/A*	**N/A***		
Total	**20/71 (28)**	**14/71 (20)**		

***** Not available, since no food samples were collected.

Table 9. List of ready-to-eat food products positive for *Listeria* species.

	Food Product	Bacterial Counts (CFU/g)	Species Isolated
Fish			
	Salmon nuggets	**< 100**	***L. monocytogenes***
	Sockeye salmon candy	**< 100**	***L. monocytogenes***
	Salmon leather	**< 100**	*L. innocua, L. welshimeri,* ***L. monocytogenes***
	Salmon leather	< 100	*L. welshimeri, L. innocua*
	Salmon leather	< 100	*L. innocua*
	Cold smoked salmon	**< 100**	***L. monocytogenes***
	Salmon candy	**< 100**	***L. monocytogenes***
	Salmon candy	**< 100**	***L. monocytogenes***
	Teriyaki smoked sablefish	**< 100**	***L. monocytogenes***
	Spring-wood smoked salmon	**< 100**	***L. monocytogenes***
	Indian candy salmon	**300**	***L. monocytogenes****, L. innocua*
	Lox whole salmon	< 100	*L. innocua*
	Lox sliced salmon Coho	400	*L. innocua*
	Prawns	< 100	*L. innocua*
	Indian candied salmon	100	*L. innocua*
	Salmon jerky	**< 100**	***L. monocytogenes***
	Cajun salmon	**> 30,000**	***L. monocytogenes***
	Shrimp meat	**< 100**	***L. monocytogenes***
	Teriyaki salmon	**> 30,000**	***L. monocytogenes***
	Honey garlic salmon	**> 30,000**	***L. monocytogenes***
Meat			
	Cheese smokie	< 100	*L. welshimeri*
	Hot pepperoni	< 100	*L. welshimeri*
	Prosciuttino salami	< 100	*L. innocua*

3.5.2 Generic *Listeria* and *L. monocytogenes* contamination of meat products

A small proportion (3/91, 3%) of RTE meat food samples were contaminated with generic *Listeria* (Table 6). None harbored *L. monocytogenes*. Non-pathogenic *L. welshimeri* was recovered from a cheese smokie sausage and a hot pepperoni sample, while *L. innocua* was found in a prosciuttino salami recovered from a facility where fewer than four food samples were collected (Table 9). In all three generic *Listeria* contaminated foods, bacterial counts were less than 100 CFU/g (Table 9).

Of the 14 meat facilities that met the food number criteria, only 2 facilities had food samples contaminated with generic *Listeria.* One facility was a slaughter facility and one belonged to the other meat processors sub-category. In each facility, a single sample was contaminated with a non-pathogenic species of *Listeria*: *L*. *welshimeri.*

3.6 Investigation of environmental and food safety practices in fish processing facilities with *L. monocytogenes* positive foods

During the course of the project, RTE food samples from five fish processing facilities tested positive for *L. monocytogenes* (Table 10 and Appendix 4). Review of BCCDC enteric disease records did not identify any reports of listeriosis cases associated with products from any of the facilities surveyed *(53)*.

Common observations in all five of the fish processing facilities where *L. monocytogenes* positive foods were identified included lack of proper cleaning and sanitation, and lack of standard operating procedures for food production.

RHAs informed BCCDC of corrective actions that were undertaken in three facilities. One facility was closed for over one month while addressing sanitation issues.

Table 10. The proportion of samples positive for *L. monocytogenes* (*L.m.*) and species of *Listeria* other than *L. monocytogenes* in five fish processing facilities that had RTE food samples positive for *L. monocytogenes*.

Case	RTE food			Environment		
	Samples taken	*L.m.* positive (%)	Other spp. of *Listeria** positive (%)	Samples taken	*L.m.* positive (%)	Other spp. of *Listeria** positive (%)
1	6	5 (83.3)	1 (16.7)	6	1 (16.7)	1 (16.7)
2	6	2 (33.3)	3 (50.0)	6	3 (50.0)	4 (66.7)
3	7	4 (57.1)	0	6	3 (50.0)	1 (16.7)
4	6	2 (33.3)	3 (50.0)	6	2 (33.3)	0
5	4	1 (25.0)	0	6	4 (66.6)	1 (16.7)

*Does not include *L. monocytogenes*.

In another facility it was discovered that the sanitizer dispenser was malfunctioning, and dispensed only water. These facilities were requested to conduct environmental and food follow-up sampling following cleaning and sanitation of the premises.

Follow-up included the requirement to have at least two successive samples of each food produced, as well as three successive environmental samples (i.e. the same surfaces that tested positive previously) test negative for the presence of *L. monocytogenes* prior to being allowed to re-institute the processing and sale of RTE fish products (Appendix 4).

Subsequent testing of products in one facility revealed high levels of contamination of a range of items, which resulted in a closure of the processor and a detailed investigation involving multiple government agencies. This processor was particularly interesting, as many environmental and equipment surfaces were tested in the facility over a one month period and only non-food contact surfaces, such as, a drain and a leg of a cart, tested positive for *L. monocytogenes*. However, on multiple occasions during the month, numerous RTE foods tested positive for *L. monocytogenes*. As contamination sources in the facility were not apparent, detailed examination of the premise design, food handling and food safety practices was undertaken. Further, the facility was renovated to address food safety issues and product flow, and personnel were trained in food safety practices. Until the health authorities were confident that the control of *L. monocytogenes* in this facility was achieved, foods produced in the facility were tested for *L. monocytogenes* prior to being released for sale.

At the three facilities described above, voluntary food recalls at the producer and retail level were instituted. Depending on the product, the recall rate was anywhere from 50% to 100% (Appendix 4). A public recall was not undertaken, in part because product was sold as a bulk item, with retailer wrapping only, thus preventing identification of manufacturers by consumers.

In all cases, the three facilities described above were allowed to process and sell RTE foods once corrective actions were implemented.

4. DISCUSSION

The ubiquitous nature of *Listeria* has been demonstrated in numerous studies. *Listeria* species, and in particular *L. monocytogenes*, have been found in food products and retail and processing environments of fish *(22,37,55)*, dairy *(29,38)* and meat facilities *(13,15,28)*. Considerable variability has been noted in the levels of contamination of food and food processing facilities with *Listeria* from region to region *(76)*. The current study demonstrates such variation in the occurrence of generic *Listeria* in dairy, fish and meat RTE products and in the facilities of the BC processors which produce them.

Discrepancies in estimates of the prevalence of *Listeria* among foods and facilities relate in part to sampling strategies as well as to which foods and facilities were sampled, and to processing and hygiene practices in those facilities. Taking this into consideration, the findings presented in the current survey may be significantly different from those reported in other Canadian regions, as well as in other countries. It is also important to note that the findings reported in this study apply specifically to the non-federally registered food processing facilities that produce RTE foods in British Columbia, and that they are a snapshot representing the situation as of mid-2009.

Although the current survey represents the *Listeria* prevalence in BC RTE facilities at one point in time, it does incorporate almost all RTE producers outside of the retail meat sector. All dairy facilities, all slaughter facilities and almost all finfish processors under provincial inspection authority were included. While only a fraction of the other (non-slaughterhouse) meat processors of RTE meat products were visited, there is no reason to believe that the RHAs which chose them would have selectively underrepresented facilities likely to be contaminated with *Listeria*. We would assert that the survey represents a valid assessment of practices and products as of mid-2009.

Further, uniformity in the collection and processing of food samples, and in procedures for swabbing the processing environment allowed for comparison of hazards in foods between the three classes of processors and among different zones of the processing facilities. The lack of a uniform facility inspection protocol however, limits our capacity to assess the relationship between environmental and food microbial test results and facility food hygiene practices; a detailed review of facility hygiene was conducted only for those facilities where contaminated product was found.

As for product sampling, the requirement to sample in facilities only after three hours of facility opening (as opposed to the start of the production day) allowed for the capture of incidents of product and environmental contamination during processing *(14,81)*. On the other hand, the collection of samples at the facilities would tend to underestimate risk to consumers that might occur through the growth of *L. monocytogenes* during shipping, handling, retail display, and as a result of consumer hygiene practices *(77)*.

As expected, a wide distribution of *Listeria* species, including *L. monocytogenes*, was observed in RTE food processing facilities in BC. The overall occurrence of generic *Listeria* in the environment of food processing facilities in the current survey was comparable to other Canadian studies *(28,30)* and to those performed in

other countries *(21,37,43,54)*. Both environmental and food samples were found positive for generic *Listeria*; however, the prevalence of generic *Listeria* in dairy and meat facilities was lower than expected. In contrast, the prevalence of generic *Listeria* in fish processing facilities was considerably higher. Of particular importance was the finding that among facility types, only in fish processing facilities was generic *Listeria* recovered from surfaces in contact with food. Relative differences in *L. monocytogenes* prevalence by facility type were even stronger. While non-food contact surfaces positive for *L. monocytogenes* were found in dairy, fish and meat categories, only in fish processing facilities were food contact surfaces and RTE foods positive for the pathogen. This points to cross contamination from non-food contact surfaces to RTE foods in fish but not meat or dairy facilities.

As for RTE foods, while 3% of meat samples were positive for generic *Listeria,* 28% of RTE fish samples harbored the bacteria. *Listeria monocytogenes,* the organism associated with human listeriosis, was recovered from fish products alone. Results for *L. monocytogenes* positive food and environmental samples were immediately reported to RHAs and BCCDC epidemiologists. During the course of the study no listeriosis illnesses were linked to foods tested in this study. Further, the genetic fingerprinting of *L. monocytogenes* strains found in the food and environmental samples did not match the strains found in reported invasive listeriosis cases during 2009 (as determined by pulsed field gel electrophoresis; *(53)*.

Findings specific to dairy, fish and meat RTE processors are discussed in the sections that follow.

4.1 Dairy processing facilities

The presence of generic *Listeria* and *L. monocytogenes* in swab samples collected in the environment of dairy processing facilities in BC was relatively low (9% and 5%, respectively). All but one of the generic *Listeria* contaminated surfaces were those not in direct contact with RTE foods, such as floor drains, areas under wash-sink, areas around and on a conveyor. Similarly, a study of dairy plants in Vermont *(58)* demonstrated a significantly higher presence of *Listeria* on floors, especially those located in coolers, compared to other non-food contact surfaces. In BC dairy facilities, *L. monocytogenes* were found mainly on floor drains. Floor drains, stainless steel steps and crates have also been identified as the main environmental sources of *L. monocytogenes* in milk processing environments in Northern Ireland *(57)* and New York *(56)*. In addition, surfaces such as conveyors and chain systems, and areas under machinery contaminated with *L. monocytogenes* have been identified as areas of concern given that cross-contamination through aerosols from pressurized washing or from employee contact may result in contamination of the machinery and foods *(70)*. Interestingly, in the current survey no contamination with *Listeria* was observed on machinery in direct contact with foods or in RTE food products, even though the bacteria were present in the environment of some of the facilities. This is in agreement with the observations made by Pritchard et al. *(70)* who suggested that, although common, the

presence of *Listeria* in the environment of a dairy processing facility does not necessarily translate into contamination of equipment having direct food contact and ultimately with product contamination, as transient *Listeria* contaminants may be eliminated effectively from dairy processing areas where special emphasis is placed on the cleaning and sanitizing of the food processing environment *(70)*.

At the time of the survey, no RTE dairy products from BC were found contaminated with generic *Listeria*. Likewise, a relatively low incidence of generic *Listeria* in Canadian cheese and non-fermented dairy products has been reported previously *(75)*. In the only known Canadian survey of domestically produced RTE cheese products reported thus far, *Listeria* were absent from 182 cheese samples *(29)*. In the same survey, of the 192 imported cheese samples, *Listeria* were present in three samples, all three manufactured by one producer *(29)*.

Even though Farber et al. *(29)* reported a low degree of contamination of domestic and imported cheese with *Listeria* spp. in Canada in their 1987 survey, they identified concerns with inadequate processing and potential for post-processing contamination, and recommended continued surveillance by regulatory agencies and industry to improve the safety of soft cheeses. These concerns were realized in the following years, as Canada experienced several listeriosis outbreaks linked to soft cheeses *(17)*.

In 2002, two separate outbreaks of listeriosis occurred in BC associated with the consumption of soft, mold ripened cheeses produced from pasteurized milk in two provincially licensed dairy facilities *(17)*. One outbreak was caused by a contaminated water supply and a breakdown in the plant water disinfection system. The cause of the other outbreak was believed to have resulted from poor hygienic practices which allowed *Listeria* to enter the facility and subsequently contaminate cheese culture solutions. As a result of these outbreaks, changes in policies regarding hygienic practices and water disinfection systems for dairy facilities were enacted in BC.

Improved manufacturing practices and programs such as HACCP, were introduced in the mid-1990s to food processing facilities, including dairy processors, across Canada with the aim of improving the safety of RTE products *(39,48)*. However, in the absence of baseline studies that would allow comparison of the prevalence of *Listeria* spp. in dairy facilities in BC during previous years to the current findings, our observation that dairy facilities and their RTE products have low levels of contamination, cannot be ascribed directly to any one of manufacturing hygiene, enhanced inspection or regulatory practices.

Provincially-licensed dairy processing facilities in BC are inspected on average two to three times a year. At least once per year, both food and environmental samples may be collected and tested for indicator organisms, *L. monocytogenes* and other pathogens as deemed appropriate by the inspector. At each inspection, in-house testing results may be requested and reviewed by the inspector. In addition, dairy facilities are required to submit monthly RTE food samples to a designated laboratory for microbiological testing.

Likewise, employees in dairy processing facilities in BC are required by regulation to be licensed as Dairy Process Workers *(36)*.

Qualification for licensing requires the completion of a recognized dairy course, such as the Dairy Processing courses offered through the BC Institute of Technology.

4.2 Fish processing facilities

Currently, fish processing facilities in BC are licensed by the species of fish they process, for example, salmon processing, trout processing, invertebrate processing. However, the license type does not discriminate between producers of RTE foods and those who prepare or eviscerate fresh fish for market. For this study, the first challenge encountered was trying to determine which license holders produced RTE foods.

Even though listeriosis outbreaks related to RTE fish products from BC have not been reported, the presence of generic *Listeria* and *L. monocytogenes* in fish processing facilities was relatively high (8/13 and 5/13 facilities, respectively) when compared to dairy (5/17 and 3/17, respectively) and meat (8/23 and 3/23 respectively) processing facilities. Nonetheless, the presence of *Listeria* in fish facilities was not unexpected as high contamination rates in fish processing facilities have been reported elsewhere *(20,21,24,37,42,47)*.

While contamination of dairy facilities was seen exclusively in the processing environment, in a substantial proportion of fish facilities, RTE products too were contaminated with generic *Listeria*, including *L. monocytogenes*. This is a clear indication that *Listeria* present in the facilities is contaminating product in many BC fish processing facilities.

While surfaces not in direct contact with RTE foods, such as drains, floors, and legs of tables and carts, were where the highest prevalence of contamination was found, in three facilities, all three types of surfaces (food contact, close-to-food contact and non-food contact) were contaminated with generic *Listeria*, as were RTE food products. Interestingly, in two facilities where *L. monocytogenes* was found in RTE foods, the bacteria were recovered only from non-food contact surfaces. The finding of non-food contact surfaces positive for *Listeria* has been reported as a sensitive indicator of the presence of *L. monocytogenes* in smoked salmon *(74,81)*.

Our finding of the association between the presence of *L. monocytogenes* in foods and the presence of *L. monocytogenes* in the environment of a processing facility has been shown elsewhere *(56,81)*. In a study of *L. monocytogenes* contamination patterns in four smoked fish processing facilities, Thimothe et al. *(81)* observed a strong positive relationship ($p<0.0001$) between *L. monocytogenes* prevalence in environmental samples and *L. monocytogenes* prevalence in finished product samples. They also reported a statistically positive relationship between *Listeria* spp. prevalence in the environment and *L. monocytogenes* prevalence in the environment ($p=0.0005$) and in finished products ($p=0.031$). While investigating risk factors associated with contamination of smoked salmon during processing, Rørvik et al. *(74)* also reported that the risk of finding *L. monocytogenes* in smoked salmon was positively associated with the presence of *L. monocytogenes* in drains (relative risk of 3.3).

Although generic *Listeria* and *L. monocytogenes* appear to be common in cold and hot smoked fish samples *(19,47)*,

the prevalence rate for *L. monocytogenes* in RTE fish products reported in the current study was notably higher than the rates reported in previous Canadian studies *(26,27)*, and in the recent report of the European Food Safety Authority (EFSA)*(21)*; current prevalence rates are however, similar to findings reported by Van Coillie et al. *(83)* for Belgium samples, and those observed by Dominguez et al. *(19)* for smoked fish and fish pâté samples in Spain.

Farber *(27)* reported the absence of *L. monocytogenes* in 196 and 150 Canadian RTE seafood products tested in 1997/1998 and 1998/1999, respectively, as part of the Canadian Food Inspection Agency's Quality Management Program. However, limited information was provided regarding the origin of the products and characteristics of facilities from which samples were collected *(27)*. In addition, only a direct plating method was used to test for *L. monocytogenes* as opposed to both the direct plating and enrichment methods applied in the current survey. The use of a direct plating method may decrease the chance of bacterial detection if microorganisms are sub-lethally injured or present in low numbers.

A 1991 Canadian study, also by Farber *(26)*, examined 113 RTE seafood products from the wholesale level for the presence of *L. monocytogenes*. Among the 113 samples tested, only 20 salmon products originated from Canada. Overall, 13% (15/113) of the tested products contained *L. monocytogenes*, which is lower than the 20% (14/71) reported here. Of 20 salmon products produced in Canada, 5 (25%) were positive for *L. monocytogenes*.

The recent summary of trends and sources of foodborne outbreaks published by the EFSA *(21)*, reported an overall prevalence of 9.8% for *L. monocytogenes* in 7,126 RTE fish products from both retail and food processing facilities in 12 European countries. As testing procedures varied from country to country, caution should be applied when interpreting the results. Further, contamination of retail product is influenced by food packaging, preparation practices, storage temperatures, the shelf-life stage of a product at the time of analysis, the effectiveness of food safety programs and the level of education and training practiced by food handlers *(20,77)*. In contrast, fish samples tested in the current survey were collected from food processing facilities at the beginning of their shelf-life, and were not exposed to shipping or handling at the retail stores. The contamination of RTE fish products with *L. monocytogenes* might have been higher had we collected samples at the end of their shelf life, or tested samples collected at the retail level *(21,77)*.

Even though *Listeria* spp. and *L. monocytogenes* have been reported in fish products worldwide, fish and fish products have rarely been involved in listeriosis outbreaks *(27,54)*. It has been suggested that as cooked fish products generally contain low levels of *L. monocytogenes* and have a short shelf life, they do not likely represent a serious health hazard *(27,73)*. Also, while in some cases high levels of contamination of fish and fish products with *L. monocytogenes* have been reported, when the low degree of consumption of RTE fish per capita is taken into account, the population health risk has been rated as low *(19)*.

In the current study, of the foods sampled, only RTE fish products were positive for *L. monocytogenes*. These microorganisms were not detected in the

tested foods produced in dairy or meat facilities. In fact, 42% (5/12) of the fish facilities contained RTE products contaminated with *L. monocytogenes.* In one particular fish processing facility, three RTE samples contained high levels of *L. monocytogenes*, which is a concern. Even though a low health risk from RTE fish contaminated with *L. monocytogenes* has been suggested elsewhere *(19,26)*, the infective dose for acquiring listeriosis infection is thought to be host and dose dependent *(25,49,76)*. While a dose of 100 organisms conveys a probability risk for infection ranging from 10^{-9} to 10^{-13}, a dose of 1,000,000 organisms increases the risk of infection to 10^{-6} to 10^{-9} *(49)*. Further, persons in vulnerable groups, such as leukemia and transplant patients, are 1000 times more susceptible to invasive listeriosis compared to healthy persons *(25,76)*. Similarly, pregnant women and their newborns are 14 times more likely to acquire invasive listerial infections compared to normal healthy population *(25,76)*. The contaminated products in BC were destined for sale to a wide population, potentially including pregnant women and immunocompromised individuals; hence, a closer look into the production of RTE fish products in BC is warranted.

It has been recognized worldwide *(32)* that the control of *L. monocytogenes* in fish and fish processing facilities is a challenge. Effective control requires facility management of conditions that lead to food contamination. For cooked products, such as hot smoked fish, a heat treatment process should be adequate to destroy *L. monocytogenes*. Following heat treatment, it is crucial that foods are handled appropriately as to avoid re-contamination prior to or during slicing, packaging, handling and sale to the consumer. For cold smoked products, cold smoking does not have a heat treatment adequate to destroy *L. monocytogenes* (smoking temperatures are below 30°C), and control of fish quality, refrigeration and strict hygiene during slicing, packaging and handling are necessary *(32)*. In this study, one cold smoked and several hot smoked fish products were contaminated with *L. monocytogenes*, indicating a control failure somewhere along the processing chain *(32)*.

4.3 Meat processing facilities

In contrast to fish, a low prevalence of generic *Listeria* was observed in the processing environment (7%) and food products (3%) of RTE meat processing facilities in BC (Table 5,Table 6).

The absence of *L. monocytogenes* in the tested RTE meat samples and its presence in only a small percentage (2%) of environmental swabs (Table 5), suggests that the control of *L. monocytogenes* in BC RTE meat facilities is adequate. However, in comparison to dairy and fish facilities where more than half of the known facilities were included in the survey, only a fraction of delis and butcher shops producing RTE meats were sampled. We are unable to assess whether samples taken from the tested facilities are truly representative of all such establishments and acknowledge that this lack of representativity may have biased the estimated prevalence rate of *Listeria* spp. in delis and butcher shops.

In the US, 3.3% of 830 dry and semidry fermented sausages and 4.4% of 1,509 sliced ham and luncheon meats sampled over a period of three years *(63)* contained *L. monocytogenes*. Similarly, 3.4% of 1,044 meat products collected over a period of

four years from retails markets in the Czech Republic contained *L. monocytogenes (41)* and 4.4% of 501 RTE meat products from the retail and meat processing facilities in Spain were found contaminated with *L. monocytogenes (13)*. In Alberta, 3 to 5% of turkey breast, beef wiener and chicken wiener samples, as well as 4% of retail fermented sausages tested by Bohaychuk et al. *(11)* contained *L. monocytogenes.*

The occurrence of non-pathogenic species of *Listeria* in RTE meat products (3%) (Table 6) and meat processing environments (5%) (Table 5) was also lower than that reported in other studies *(10,11)*.

However, the prevalence rates reported in different studies need to be compared with caution, as certain limitations, such as results obtained from regulatory product-testing programs which include testing over a longer period of time and samples obtained at both the retail and processing environments, may significantly impact the reported rates. Further, the type of product and its manufacturing process, product composition, and intrinsic characteristics have been known to play a role in the prevalence of *L. monocytogenes* in RTE meats *(12,64)*.

As suggested by Lianou and Sofos *(64)*, while extensive measures have been applied in food facilities to reduce *L. monocytogenes* in the processing environment, the same level of control is generally not seen in retail establishments. Similar to food processing facilities, retail establishments with high *Listeria* contamination in the environment and equipment, especially slicers and work-tables, pose a high risk for cross-contamination of food *(77)*.

The relatively low prevalence rate for generic *Listeria* and *L. monocytogenes* in meat processing facilities in BC may be due to greater awareness of the potential for *L. monocytogenes* contamination in meat, as a result of the recent Canada-wide listeriosis outbreak involving processed meats *(6)*.

4.4 Using facility swab samples and food samples positive for generic *Listeria* as a means to screen for the presence of *L. monocytogenes* in foods

CFIA mandates the use of swabs for assessment of processing environments and foods produced as a means of ensuring *L. monocytogenes*-free product *(14)*. As testing for generic *Listeria* is quicker and cheaper to perform, results were reviewed to assess the validity of culturing for generic *Listeria* as a means to identify the risk of *L. monocytogenes* in food products. The contingency table approach presented here is commonly used in assessing the performance of screening tests.

Our findings indicate that in the current BC context, when generic *Listeria* are found in the processing environment, 28% of facilities will have *L. monocytogenes* in at least one food product; however, when generic *Listeria* are not found in the environment there is certainty (100%) that *L. monocytogenes* is absent from the facility's food (Table 11). This analysis speaks to the value of environmental surface swabbing and analyzing the swabs for generic *Listeria* as a way to predict with confidence that food produced in the facility is unlikely to harbor *L. monocytogenes.*

Table 11. Contingency table for generic *Listeria* found in at least one environmental swab sample versus *L. monocytogenes* found in at least one food sample, by facility (facilities that met both inclusion criteria included).

		L. monocytogenes in food samples			
		Yes	No	Total	
Generic *Listeria* on environmental swabs	Yes	5	13	18	**PPV***: 28%
	No	0	25	25	**NPV**†: 100%
	Total	5	38	43	

*Positive predictive value: true positive samples divided by the sum of true positive and false positive samples (here, 5 / (5+13) = 0.28) expressed as %.
†Negative predictive value: true negative samples divided by the sum of false negative and true negative samples (here, 25 / (0+25) = 0.96) expressed as %.

Looking at the predictive accuracy of culturing for generic *Listeria* in food, 63% of facilities where a food sample tests positive for generic *Listeria*, will in fact have food contaminated with *L. monocytogenes* (Table 12).

As testing for generic *Listeria* is quicker and cheaper than testing for *L. monocytogenes,* the survey shows that in the BC context, culturing for generic *Listeria* offers an effective screening option.

Table 12. Contingency table for generic *Listeria* found in ANY food sample versus *L. monocytogenes* found in ANY food sample, by facility (facilities that met food inclusion criteria included).

		L. monocytogenes in food samples			
		Yes	No	Total	
Generic *Listeria* in food samples	Yes	5	3	8	**PPV***: 63%
	No	0	35	35	
	Total	5	38	43	

*Positive predictive value

Swabbing the processing environment for bacterial analysis is an important tool for industry and for Environmental Health Officers to assess the level of hazard and degree of control of *Listeria* in food processing environments. Swabbing non-food contact surfaces, such as drains, prior, during and after food processing to test for the presence of generic *Listeria* can assess whether these bacteria are present in the facility, and whether the potential of the food contamination during production exists, as well as whether adequate cleaning and sanitation takes place following the food production. Tests of close-to-food and food-contact surfaces further assess the risk that *Listeria* may contaminate foods during food processing.

4.5 Management of hygiene in facilities with *L. monocytogenes* positive foods

All facilities surveyed in this project were subject to provincial inspection, which they continue to receive.

As no provincial guidelines currently exist for appropriate actions to be taken when an environmental surface or a food product is found positive for *L. monocytogenes* or other *Listeria* spp. in facilities producing RTE foods, management recommendations were drafted during the course of the project. Uncertainty about how best to interpret and implement guidance from other agencies (i.e. the Health Canada policy and activities in federally inspected establishments) was an obvious area requiring improvement for future consensus and action by provincial regulatory authorities.

One of the concerns noted during follow-up investigation of a contaminated RTE food (smoked salmon nuggets) was that these products are often sold to

consumers in unlabelled bags. In the case of contaminated smoked salmon nuggets, these products were first sold to retailers in bulk packages who displayed them on ice.

Generally, when these products are displayed, the name of the facility where the product originated may or may not be present on the tag information. Even if *Listeria* were identified in a RTE product, it is questionable whether the public would be able to recognize where the food was manufactured, and thus respond appropriately to specific recall advice. In addition, consumers who purchase these products may not be aware that many of them are categorized as potentially hazardous foods (i.e. foods with pH above 4.6 and water activity above 0.85). Also, consumers may not be aware that these products should be refrigerated in order to limit the growth of bacteria such as *L. monocytogenes.* This is of particular concern to highly vulnerable consumers, such as those pregnant women, immunocompromised persons, and the elderly, for whom infection with *L. monocytogenes* can lead to fatal listeriosis.

Another concern with bulk product is the potential for cross contamination with other products at the retail level. For example, in retail display cases, contaminated product from one batch may be mixed with uncontaminated product leading to further RTE food listerial contamination. Shared tongs used to dispense these products are also a likely vehicle for cross contamination.

As mentioned, one of the limits of this study was that RTE foods were not tested at other points in the food chain before reaching the consumer. Also, during collection of samples for this survey, no concurrent objective assessment of the facility was performed, such as assessments of food handling and production practices, cleaning and sanitation, and employee hygiene, to establish facility risk. This information would have been valuable to compare the presence of *Listeria* in a facility to an objective assessment of operating practices. Other pathogens of interest, such as *Salmonella*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* were not included in the test panel to limit costs.

A success of the survey, although not its primary goal, was the set of actions taken to ensure food safety measures in those fish processing facilities found to be producing foods contaminated with *L. monocytogenes*. Appropriate public health actions by processors, Regional Health Authorities and other regulatory agencies were taken to promote facility hygiene and so protect public health. Primarily, and in response to its objectives, the survey revealed the previously unidentified widespread degree of contamination by *L. monocytogenes* of BC fish processing facilities under provincial inspection authority, and emphasized the importance of sampling for specific pathogens during routine inspection of facilities in order to improve the safety of RTE foods in British Columbia.

5. CONCLUSIONS AND RECOMMENDATIONS

In BC facilities under provincial inspection authority, *L. monocytogenes* was recovered from 7 of 204 food- or close-to food contact surfaces and in one or more RTE products from 5 of the 53 visited facilities. Statistical analysis of the proportion of food samples and swabs positive for *Listeria*, by food category, calculated both by facility and by samples from all facilities combined, gave consistent results: in RTE food processing facilities under BC provincial inspection authority, fish processors are where *Listeria* is found, particularly on food contact surfaces, and in RTE products. *Listeria monocytogenes* was not cultured from RTE dairy or meat products and was found at low levels and only on surfaces not in direct food contact in these facilities. In contrast, BC fish processing facilities were commonly contaminated with generic *Listeria*, and in 2 of the 13 fish processing facilities visited, *Listeria monocytogenes* was recovered from food contact surfaces. *Listeria monocytogenes* was recovered from food products in 5 of 12 RTE fish facilities, in some cases at high levels.

A correlation between the level of hygiene practiced in a facility and the prevalence of *L. monocytogenes* has been demonstrated in many studies *(38,56,58,61)*, emphasizing the need not only for stringent but also for continuous control strategies. The absence of generic *Listeria* in the great majority of BC's dairy and meat RTE processors shows that the bacteria can be kept at low levels in RTE facilities. On the other hand, in the majority of fish processing facilities where *L. monocytogenes* was recovered from RTE food samples, inadequate sanitation and/or the lack of rigorous food hygiene practices were observed.

In Canada, federally registered food (including fish) processing facilities are subject to environmental and end-product testing for generic *Listeria* and/or *L. monocytogenes*; however, this level of inspection is not required nor practiced in most non-federally registered food processing facilities. Instead, on the provincial level, the food industry bears the primary responsibility for the production of safe foods *(52)*.

The current study suggests that a combination of monitoring and validation of food safety practices, whether through periodic environmental sampling, end product testing, more rigorous inspection, or a combination of these, is warranted in RTE food processing facilities in BC, especially in RTE fish processing facilities.

Recent outbreaks related to contaminated processed meats and cheeses have raised questions concerning the efficacy of the food safety system in Canada, on both provincial and federal levels. In the 2008 Canada-wide deli-meat listeriosis outbreak, longitudinal testing of environmental swabs revealed ongoing contamination of meat processing lines with generic *Listeria* prior to the onset of the outbreak. A post-mortem of the outbreak highlighted the importance of following trends in microbial analyses of environmental samples as an early indicator of the potential for contamination of RTE products *(85)*.

These events have highlighted the need for more targeted inspections of RTE processors and for more baseline studies to evaluate the occurrence and spread of pathogens, such as *L. monocytogenes,* in

food processing environments and their products *(52,85)*.

Follow-up activities underway at BCCDC

1. With the National Microbiology Laboratory and PHSA Laboratories, further testing of the positive *L. monocytogenes* isolates is in progress, based on serological (Serotyping) and genetic (Pulsed Field Gel Electrophoresis) methods to provide additional information regarding the properties of the isolates, and to provide links to any reported human cases, from which cultures are subjected to the same fingerprinting techniques.
2. A fish inspection course is being developed for provincial inspectors (including Environmental Health Officers and Fishery Officers) to focus on critical processes in fish manufacturing.
3. Collaborative efforts between BC provincial government authorities and the Canadian Food Inspection Agency are currently in progress to develop a meat inspection course for Environmental Health Officers to focus on critical processes in RTE meat production.

Recommendations

1. Remind vulnerable populations in BC of the risk associated with consumption of food products such as soft cheese, deli-meats and smoked fish. In particular, until levels of *L. monocytogenes* in BC product drop, or until a province-wide testing and labeling program can be put in place, pregnant women, immunocompromised individuals and the elderly should be advised of the risks associated with the high prevalence of *L. monocytogenes* in ready-to-eat smoked fish products. These food products have not been emphasized in previous educational campaigns or in media coverage of listeriosis outbreaks associated with deli meats and soft cheeses. These actions would involve collaboration with fish processors and distributors, BC Ministry of Agriculture and Lands, the Provincial Health Officer, the Ministry of Health Services, and the BC Medical Association.
2. Propose an evidence–based sampling guideline for industry and government for effective monitoring of *Listeria* spp. and *L. monocytogenes* (and other foodborne pathogens) to include: environments and products to sample, sampling procedures, frequency of sampling, cost of sampling, and recommended follow-up actions.
3. Enhance the training for food inspectors on how to test for and control *Listeria* spp. in processing environments.
4. Explore the use of Hazard Analysis Critical Control Points (HACCP) and other hazard identification programs in dairy, meat and fish processing plants along with estimation of the costs and effectiveness of these programs in BC and jurisdictions elsewhere.
5. It is clear from the results of this study that fish processing facilities producing ready-to-eat foods require special attention. The purpose of both further research and of an enhanced inspection regime would be to improve food safety for consumers of RTE fish products. Specific activities and recommendations for fish processing facilities include:
   a. Identification of all provincially licensed fish processing facilities

currently producing RTE foods in order to better track output and performance.

b. Assessment of HACCP programs and other control measures in place in fish processing facilities, in conjunction with a follow up microbial assessment survey of facilities in BC that produce RTE fish/seafood products. A more extensive survey combining environmental swabs, product testing, and a detailed hazard assessment (including facility sanitation, employee hygiene, attention given to critical control points along the processing chain, and adequacy of monitoring and verification procedures) would be related to environmental and food microbial testing results. This approach would allow objective assessment of the effectiveness of control measures. Effective means to audit and track facility hygiene would be the outcome.
c. Encourage research into how *Listeria* enters and spreads through the processing environment of smaller RTE producers.
d. In the case of facilities where *Listeria* is identified, document control measures where implemented, and their impact on the presence of *Listeria* in the processing environment and in food products. This would allow the optimal incorporation of practice-based learning into policies and procedures.
e. Establish a working group of stakeholders including industry, BC Ministry of Agriculture and Lands, BC Ministry of Health Services, BC Regional Health Authorities and BCCDC to review the results of this and subsequent surveys of fish processing facilities, seek out system improvements and suggest future policy.

6. LITERATURE CITED

1. Anonymous. 1996. Fish Inspection Act. Chapter 148. Available at: http://www.bclaws.ca/EPLibraries/bclaws_new/document/ID/freeside/00_96148_01. Accessed 25 May 2010.
2. Anonymous. 1996. Milk Industry Act. [RSBC 1996] CHAPTER 289. Available at: http://www.bclaws.ca/EPLibraries/bclaws_new/document/ID/freeside/00_96289_01. Accessed 30 April 2010.
3. Anonymous. 1999. Food Premises Regulation. Public Health Act. B.C. Reg. 210/99, O.C. 774/99 Available at: http://www.bclaws.ca/EPLibraries/bclaws_new/document/ID/freeside/11_210_99. Accessed 30 April 2010.
4. Anonymous. 2004. Meat Inspection Regulation. Food Safety Act. B.C. Reg. 349/2004, O.C. 798/2004. Available at: http://www.bclaws.ca/EPLibraries/bclaws_new/document/ID/freeside/10_349_2004#section10. Accessed 30 April 2010.
5. Anonymous. 2009. British Columbia annual summary of reportable diseases for 2008 - Listeriosis. British Columbia Centre for Disease Control. Available at: http://www.bccdc.ca/NR/rdonlyres/59BFCFBB-933D-4337-9305-E3E5FF30D272/0/EPI_Report_CDAnnual2008_20090806.pdf. Accessed 19 November 2009.
6. Anonymous. 2009. *Listeria monocytogenes* outbreak. Health Issues. Public Health Agency of Canada. Available at: http://www.phac-aspc.gc.ca/alert-alerte/listeria/listeria_2009-eng.php. Accessed 19 November 2009.
7. Anonymous. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food --- 10 States, 2008. *MMWR Morb. Mortal. Wkly. Rep.* 58(13): 333-337.
8. Anonymous. 2010. British Columbia annual summary of reportable diseases for 2009 - Listeriosis. British Columbia Centre for Disease Control. Available at: http://www.bccdc.ca/NR/rdonlyres/5F0507F1-FA6A-41EF-9F84-7C530A65ADB3/0/EPI_SR_CDAnnual2009_20100819.pdf. Accessed 5 October 2010.
9. Anonymous. 2010. Food Safety Act. Chapter 28. [SBC 2002]. Available at: http://www.bclaws.ca/EPLibraries/bclaws_new/document/ID/freeside/00_02028_01. Accessed 25 May 2010.
10. Barros, M. A. F., L. A. Nero, L. C. Silva, L. d'Ovidio, F. A. Monteiro, R. Tamanini, R. Fagnani, E. Hofer and V. Beloti. 2007. *Listeria monocytogenes*: Occurrence in beef and identification of the main contamination points in processing plants. *Meat Sci.* 76(4): 591-596.
11. Bohaychuk, V. M., G. E. Gensler, R. K. King, K. I. Manninen, O. Sorensen, J. T. Wu, M. E. Stiles and L. M. McMullen. 2006. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. *J. Food Prot.* 69(9): 2176-2182.
12. Buncic, S. and S. M. Avery. 2004. Microbiological safety of meat | *Listeria monocytogenes*, p. 804-814. *In* J. Werner Klinth (ed.), Encyclopedia of meat sciences, vol. 1-4. Elsevier, Oxford.
13. Cabedo, L., L. Picart i Barrot and A. eixidó i Canelles. 2008. Prevalence of *Listeria monocytogenes* and *Salmonella* in ready-to-eat food in Catalonia, Spain. *J. Food Prot.* 71(4): 855-859.

14. Canadian Food Inspection Agency. 2009. Control measures for *Listeria* in ready-to-eat (RTE) meat products. Meat Hygiene Directive. Available at: http://www.inspection.gc.ca/english/fssa/meavia/man/direct/2009/direct1e.shtml. Accessed 25 November 2009.
15. Chao, G., Y. Deng, X. Zhou, Q. Xu, X. Qian, L. Zhou and B. Zhu. 2006. Prevalence of *Listeria monocytogenes* in delicatessen food products in China. *Food Control* 17(12): 971-974.
16. Charlton, B. R., H. Kinde and L. H. Jensen. 1990. Environmental survey for *Listeria* species in California milk processing plants. *J. Food Prot.* 53(3): 198-201.
17. Clark, C. G., J. Farber, F. Pagotto, N. Ciampa, K. Doré, C. Nadon, K. Bernard and L.-K. Ng. 2010. Surveillance for *Listeria monocytogenes* and listeriosis, 1995-2004. *Epidemiol. Infect.* 138(04): 559-572.
18. Dharmarha, V. 2008. A focus on *Listeria monocytogenes* -- updated version. Pathogens and Contaminants. *Listeria monocytogenes*. Food Safety Research Information Office, United States Department of Agriculture, National Agricultural Library. Available at: http://fsrio.nal.usda.gov/document_fsheet.php?product_id=221. Accessed 18 November 2009.
19. Dominguez, C., I. Gomez and J. Zumalacarregui. 2001. Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pâté sold in Spain. *J. Food Prot.* 64(12): 2075-2077.
20. EFSA. 2007. Scientific Opinion of the Panel on Biological Hazards on a request from the European Comission on request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness. *EFSA Journal* [serial online] 599: 1-42. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/599.pdf. Accessed 5 March 2010.
21. EFSA. 2010. The community summary report on trends and sources of zoonoses, zoonotic zgents, antimicrobial resistance and foodborne outbreaks in the European Union in 2008. *EFSA Journal* 1496: 1-288.
22. Eklund, M. W., F. T. Poysky, R. N. Paranjpye, L. C. Lashbrook, M. E. Peterson and G. A. Pelroy. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *J. Food Prot.* 58: 502-508.
23. El-Shenawy, M. A. 1998. Sources of *Listeria* spp. in domestic food processing environment. *Int. J. Environ. Health Res.* 8(3): 241-251.
24. Embarek, P. K. B. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. *Int. J Food Microbiol.* 23(1): 17-34.
25. FAO and WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary. Microbiological risk assessment series No. 4. Available at: http://www.who.int/foodsafety/publications/micro/en/mra4.pdf. Accessed 5 October 2010.
26. Farber, J. M. 1991. *Listeria monocytogenes* in fish products. *J. Food Prot.* 54(12): 922-924,.
27. Farber, J. M. 2000. Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. *Int. J. Food Microbiol.* 62(3): 247-251.
28. Farber, J. M. and E. Daley. 1994. Presence and growth of *Listeria monocytogenes* in naturally-contaminated meats. *Int. J Food Microbiol.* 22(1): 33-42.

29. Farber, J. M., M. A. Johnston, U. Purvis and A. Loit. 1987. Surveillance of soft and semi-soft cheeses for the presence of *Listeria* spp. *Int. J Food Microbiol.* 5(2): 157-163.
30. Farber, J. M., F. Pagotto and C. Scherf. 2007. Incidence and behavior of *Listeria monocytogenes* in meat products, p. 503-570. *In* E. T. Ryser and E. H. Marth (eds.), *Listeria*, listeriosis and food safety, vol. 3. CRC Press, Boca Raton, FL.
31. Farber, J. M. and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Mol. Biol. Rev.* 55(3): 476-511.
32. Food and Agriculture Organization of the United Nations; Fisheries and Aquaculture Department. 1999. Report of the FAO expert consultation on the trade impact on *Listeria* in fish products. FAO Fisheries Report No. 604. Available at: http://www.fao.org/docrep/003/x3018e/x3018e00.htm. Accessed 11 January 2010.
33. Food Directorate. Health Products and Food Branch. Health Canada. 2004. Policy on *Listeria monocytogenes* in ready-to-eat foods. Available at: http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/legislation/policy_listeria_monocytogenes_politique_toc-eng.pdf. Accessed 15 March 2010.
34. Food Directorate. Health Products and Food Branch. Health Canada. 2010. DRAFT Policy on *Listeria monocytogenes* in Ready-to-Eat Foods. Available at: http://www.hc-sc.gc.ca/fn-an/consultation/init/_listeria/draft-ebauche-eng.php. Accessed 12 May 2010.
35. Food Directorate. Health Products and Food Branch. Health Canada. 2010. Policy on *Listeria monocytogenes* in ready-to-eat foods. Available at: http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2010-eng.php. Accessed
36. Food Protection Services. 2005. Dairy processing plants. Plant construction, equipment and operation standards to qualify for licensing in British Columbia. Available at: http://www.bccdc.ca/NR/rdonlyres/41592F93-B78C-40E7-A929-44613FB16C81/0/DairyProcessingPlants.pdf. Accessed 22 January 2010.
37. Food Standards Agency. 2008. A microbiological survey of retail smoked fish with particular reference to the presence of *Listeria monocytogenes*. Available at: http://www.foodbase.org.uk/results.php?f_report_id=261. Accessed 4 February 2010.
38. Fox, E., T. O'Mahony, M. Clancy, R. Dempsey, M. O'Brien and K. Jordan. 2009. *Listeria monocytogenes* in the Irish dairy farm environment. *J. Food Prot.* 72: 1450-1456.
39. Gagnon, B., V. McEachern and S. Bray. 2000. The role of the Canadian government agency in assessing HACCP. *Food Control* 11(5): 359-364.
40. Garrido, V., L. Torroba, I. García-Jalón and A. I. Vitas. 2008. Surveillance of listeriosis in Navarre, Spain, 1995-2005 - epidemiological patterns and characterisation of clinical and food isolates. *Euro. Surveill.* [serial online] 13(49): pii=19058. Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19058. Accessed 23 November 2009.
41. Gelbíčová, T. and R. Karpíšková. 2009. Occurrence and characterization of *Listeria monocytogenes* in ready-to-eat food from retail market in the Czech Republic. *Czech J. Food Sci.* 27(2): S2-3-7.
42. Gianfranceschi, M., A. Gattuso, S. Tartaro and P. Aureli. 2003. Incidence of *Listeria monocytogenes* in food and environmental samples in Italy between

1990 and 1999: Serotype distribution in food, environmental and clinical samples. *Eur. J. Epidemiol.* 18(10): 1001-1006.
43. Gombas, D. E., Y. Chen, R. S. Clavero and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J. Food Prot.* 66: 559-569.
44. Gouin, E., J. Mengaud and P. Cossart. 1994. The virulence gene cluster of *Listeria monocytogenes* is also present in *Listeria ivanovii*, an animal pathogen, and *L. seeligeri*, a nonpathogenic species. *Infect. Immun.* 62: 3550-3553.
45. Goulet, V., C. Jacquet, P. Martin, V. Vaillant, E. Laurent and H. de Valk. 2006. Surveillance of human listeriosis in France, 2001-2003. *Euro. Surveill.* [serial online] 11(6): pii=629. Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=629. Accessed 10 November 2009.
46. Graves, L. M., L. O. Helsel, A. G. Steigerwalt, R. E. Morey, M. I. Daneshvar, S. E. Roof, R. H. Orsi, E. D. Fortes, S. R. Millilo, H. C. den Bakker, M. Wiedmann, B. Swaminathan and B. D. Sauders. 2009. *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. *Int.l J. Syst. Evol. Microbiol.*(60): 1280.
47. Hartemink, R. and F. Georgsson. 1991. Incidence of *Listeria* species in seafood and seafood salads. *Int. J Food Microbiol.* 12(2-3): 189-195.
48. Herath, D. and S. Henson. 2006. Does Canada need mandatory HACCP? Evidence from the Ontario food processing sector. *Can. J. Agr. Econ.* 54(4): 443-459.
49. Heymann, D. L. (ed.) 2008. Control of communicable diseases manual. American Public Health Association Press, Washington, DC.
50. Ho, A. J., V. R. Lappi and M. Wiedmann. 2007. Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility. *J. Dairy Sci.* 90(5): 2517-2524.
51. Holah, J. T., J. Bird and K. E. Hall. 2004. The microbial ecology of high-risk, chilled food factories; evidence for persistent *Listeria* spp. and *Escherichia coli* strains. *J. Appl. Microbiol.* 97: 68-77.
52. Holley, R. A. 2010. Smarter inspection will improve food safety in Canada. *Can. Med. Assoc. J.* 182: 471-473.
53. Isaac-Renton, J. L. and PHSA Laboratories. 2010. Study. Vancouver.
54. Jinneman, K. C., M. M. Wekell and M. W. Eklund. 2007. Incidence and behavior of *Listeria monocytogenes* in fish and seafood, p. 617-653. *In* E. T. Ryser and E. H. Marth (eds.), *Listeria*, listeriosis and food safety, vol. 3. CRC Press, Boca Raton, FL.
55. Johansson, T., L. Rantala, L. Palmu and T. Honkanen-Buzalski. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *Int. J. Food Microbiol.* 47(1-2): 111-119.
56. Kabuki, D. Y., A. Y. Kuaye, M. Wiedmann and K. J. Boor. 2004. Molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants. *J. Dairy Sci.* 87(9): 2803-2812.
57. Kells, J. and A. Gilmour. 2004. Incidence of *Listeria monocytogenes* in two milk processing environments, and assessment of *Listeria monocytogenes* blood agar for isolation. *Int. J Food Microbiol.* 91(2): 167-174.
58. Klausner, R. B. and C. W. Donnelly. 1991. Environmental sources of *Listeria* and *Yersinia* in Vermont dairy plants. *J. Food Prot.* 54: 607-611.
59. Koch, J. and K. Stark. 2006. Significant increase of listeriosis in Germany - Epidemiological patterns 2001-2005. *Euro. Surveill.* [serial online] 11(6): pii=631.

Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=631. Accessed 10 November 2009.

60. Kovačević, J., V. M. Bohaychuk, P. Romero Barrios, G. E. Gensler, D. L. Rolheiser and L. M. McMullen. 2009. Evaluation of environmental sampling methods and rapid detection assays for recovery and identification of *Listeria* spp. from meat processing facilities. *J. Food Prot.* 72(4): 696-701.
61. Kozak, J., T. Balmer, R. Byrne and K. Fisher. 1996. Prevalence of *Listeria monocytogenes* in foods: Incidence in dairy products. *Food Control* 7(4-5): 215-221.
62. Leclercq, A., D. Clermont, C. Bizet, P. A. D. Grimont, A. Le Fleche-Mateos, S. M. Roche, C. Buchrieser, V. Cadet-Daniel, A. Le Monnier, M. Lecuit and F. Allerberger. 2009. *Listeria rocourtiae* sp. nov. *Int. J. Syst. Evol. Microbiol.*(60): 2210.
63. Levine, P., B. Rose, S. Green, G. Ransom and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *J. Food Prot.* 64(8): 1188-1193.
64. 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: 2172-2198.
65. Lin, C.-M., K. Takeuchi, L. Zhang, C. B. Dohm, J. D. Meyer, P. A. Hall and M. P. Doyle. 2006. Cross-contamination between processing equipment and deli meats by *Listeria monocytogenes*. *J. Food Prot.* 69(1): 71-79.
66. Little, C. L., S. K. Sagoo, I. A. Gillespie, K. Grant and J. McLauchlin. 2009. Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in selected retail ready-to-eat foods in the United Kingdom. *J. Food Prot.* 72: 1869-1877.
67. Møretrø, T. and S. Langsrud. 2004. *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. *Biofilms* 1(02): 107-121.
68. Pagotto, F., E. Daley, J. Farber and D. Warburton. 2001. Isolation of *Listeria monocytogenes* from all food and environmental samples. MFHPB-30, p.1-15. *In* D. Warburton (ed.) Compendium of Analytical Methods. Health Canada Methods of Microbiological Analysis of Foods, vol. 2. Polyscience Publications Inc., Montreal.
69. Pagotto, F., E. Daley and J. M. Farber. 2002. Enumeration of *Listeria monocytogenes* in foods. MFLP-74, p.1-5. *In* D. Warburton (ed.) Compendium of Analytical Methods. Health Canada Methods of Microbiological Analysis of Foods, vol. 2. Polyscience Publications Inc., Montreal.
70. Pritchard, T. J., K. J. Flanders and C. W. Donnelly. 1995. Comparison of the incidence of *Listeria* on equipment versus environmental sites within dairy processing plants. *Int. J Food Microbiol.* 26(3): 375-384.
71. Public Health Agency of Canada. 2010. Update to 2008 *Listeria monocytogenes* case numbers. Available at: http://www.phac-aspc.gc.ca/alert-alerte/listeria/listeria_20100413-eng.php. Accessed 24 June 2010.
72. Rocourt, J., H. Hof, A. Schrettenbrunner, R. Malinverni and J. Bille. 1986. Acute purulent *Listeria seeligeri* meningitis in an immunocompetent adult. *Schweiz. Med. Wochenschr.* 116(8): 248.
73. Rørvik, L. M. 2000. *Listeria monocytogenes* in the smoked salmon industry. *Int. J Food Microbiol.* 62(3): 183-190.

74. Rørvik, L. M., E. Skjerve, B. R. Knudsen and M. Yndestad. 1997. Risk factors for contamination of smoked salmon with *Listeria monocytogenes* during processing. *Int. J Food Microbiol.* 37(2-3): 215-219.
75. Ryser, E. T. 2007. Incidence and behavior of *Listeria monocytogenes* in cheese and other fermented dairy products, p. 405-501. *In* E. T. Ryser and E. H. Marth (eds.), *Listeria*, listeriosis and food safety, vol. 3. CRC Press, Boca Raton, FL.
76. Ryser, E. T. and E. H. Marth (eds). 2007. *Listeria*, listeriosis and food safety. CRC Press, Boca Raton, FL.
77. 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: 2337-2349.
78. 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. *N Engl J Med* 308(4): 203-206.
79. Suslow, T. and L. Harris. 2000. Guidelines for controlling *Listeria monocytogenes* in small- to medium-scale packing and fresh-cut operations. Publication 8015. University of California. Division of Agriculture and Natural Resources. (ISBN 978-1-60107-195-8). Available at: http://ucanr.org/freepubs/docs/8015.pdf. Accessed 30 June 2010.
80. Swaminathan, B. and P. Gerner-Smidt. 2007. The epidemiology of human listeriosis. *Microbes Infect.* 9(10): 1236-1243.
81. Thimothe, J., K. K. Nightingale, K. Gall, V. N. Scott and M. Wiedmann. 2004. Tracking of *Listeria monocytogenes* in smoked fish processing plants. *J. Food Prot.* 67(2): 328-341.
82. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J. Food Prot.* 65(4): 709-725.
83. Van Coillie, E., W. Hadewig, H. Marc, H. Lieve and R. Nancy. 2004. Prevalence and typing of *Listeria monocytogenes* in ready-to-eat food products on the Belgian market. *J. Food Prot.* 67(11): 2480-2487.
84. Vazquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* 14(3): 584-640.
85. Weatherill, S. 2009. Report of the indipendent investigator into the 2008 listeriosis outbreak. Listeriosis Investigative Review. Agriculture and Agri-Food Canada. Available at: http://www.listeriosis-listeriose.investigation-enquete.gc.ca/index_e.php?s1=rpt&page=tab. Accessed 18 November 2009.

APPENDIX 1

Sample Collection Procedure

This page intentionally left blank.

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Standard Operating Procedure for Collection and Handling of Environmental and Food Samples for *Listeria* spp. Testing

Prepared By:

Food Protection Services
Issue Date: July 15, 2009
604.707.2440

Food Protection Services, Environmental Health Services Div
Main Floor 655 12th Ave W, Vancouver BC V5Z 4R4
www.bccdc.ca
Tel 604.707.2440 | Fax 604.707.2441

Scope and Application:

This document describes the methodology used to collect and handle ready-to-eat food products and samples from the environment of food processing facilities in order to test for *Listeria* species.

Definitions

Aseptic technique: Refers to procedures used by microbiologists to prevent microbial contamination of themselves, which may result in infection, contamination of the environment they are working in, and contamination of the specimen they are working on.

Biosafety: Relates to prevention and precautions deemed necessary to reduce the risk to people, animals and environment caused by infectious materials. Biosafety procedures may include the use of protective clothing and equipment, proper discard of waste, working only in designated areas etc.

Health and Safety:

Wear protective clothing and equipment including lab coat, steel-toe safety boots, hair net and hard hat when collecting samples in food processing environments.

All samples collected should be treated as if they contain pathogenic microorganisms and they should be handled and discarded with care, in accordance to biosafety practices. *Listeria monocytogenes* is a risk group 2 organism and materials and samples contaminated with this microorganism need to be autoclaved prior to disposal, or discarded into special biohazardous waste.

Cautions:

Follow appropriate guidelines during samples collection.

Strict adherence to the protocol is necessary for the validity of the test results.

Special Apparatus and Materials:

1. Sponge sampling kit
    - Pre-moistened sponges
    - Whirl-pak® bags
2. Permanent felt pen or marker
3. Lab forms
4. Medium to large plastic bags
5. Cooler with ice packs
6. Appropriate clothing

    e.g. lab coat, steel-toe safety boots, hair net and hard hat.

NOTE: Have the following materials on hand for testing

- Permanent felt marker for labelling samples
- An extra bag to dispose of garbage during sampling as some of the environmental sampling “kits” have disposable forceps, inner bags etc.

- A carry bag you can put over your shoulder for all testing materials or a convenient container.
- Lab/sampling forms

Collection of Environmental Samples

1. Prior to sampling, label the outside of the bag with the appropriate information that will identify the area being swabbed, including **Name of the facility, sampling site, type of location, date and time of collection.**
2. Wash your hands before you start with the sample collection.
3. Separate the whirl-pak® bag from the sponge package.
4. Hold the whirl-pak® bag in the hand which you will not use for sampling. If both hands are required for sampling, place the whirl-pak® bag into the pocket of the clean lab coat.
5. Open the sponge package.
6. Aseptically remove the sponge from the package holding onto the handle of the sterile forceps provided with the swab.
7. Rub sponge firmly and thoroughly over the surface to be sampled.
8. Size of the swabbing area should be 30 cm by 30 cm (e.g. size of a standard ruler) whenever possible.
9. Swab the area 5 times from the bottom to the top using one side of the sponge and 5 times from left to the right using the other side of the sponge.

   For surfaces which are difficult to reach or swab in this manner due to their shape, rub the area as thoroughly as possible.
10. Remove the top layer of the whirl-pak® bag and pull it open, aseptically, using the white straps on the top of the bag.
11. Transfer the sponge into the whirl-pak® bag, carefully, so that it does not come into contact with the outside of the bag, and release the sponge from the forceps.
12. With minimal air trapped inside the bag, close it and roll the ends of the whirl-pak® bag.
13. Use **NEW** sampling package kit for each sample.
14. Collect **SIX** environmental samples in **READY-TO- EAT product handling areas**:
    a. Two samples from **non-food contact surfaces** listed in Table 1.
    b. Two samples from **close to food contact surfaces** listed in Table 1.
    c. Two samples from **food contact surfaces** listed in Table 1.
15. Fill out **ONE** "*Listeria* in food processing establishments" sample tracking form for **each facility** (this is a multi-sample form; all samples, environmental and food, can be listed onto this single form). Ensure that **ALL** the fields are filled-out.
16. Place all the samples from a facility together in a plastic bag with the corresponding sample tracking form.
17. Use separate bags for each facility.
18. Place the bag containing samples and the corresponding sample tracking form into a clean and sanitized cooler with clean and sanitized ice packs.

19. Keep the samples at refrigeration temperatures (i.e. 0 to 4°C) and bring them or ship the cooler to the laboratory as soon as possible or **within 24 hours of sample collection**.
20. Do **NOT** freeze the samples.

Food Sample Collection

1. Collect **6 ready-to-eat food samples ONLY** from each food processing plant.
2. Collect at least 150 g of intact, whole food sample in the form in which it will be sold or distributed.
3. Samples should include different types of foods processed in the plant on the date of the environmental sampling or processed not more than two days prior to sampling.

   Note: If food samples are not available on the scheduled collection day or older than two days, reschedule the collection date and do **NOT** collect environmental samples.
4. If a facility produces only one type of product, collect the random samples of the same product.

   If possible, choose samples produced on different lines, products with different lots or codes etc.
5. Place each food sample into a separate whirl-pak® or clean plastic bag.
6. Make sure that the top of the bag is adequately closed.
7. Label the outside of the bag with the specific information that will identify the sample, including **name of the facility**, **type of food** (including specific details, such as the way it is processed or specific ingredients, which will separate it from other similar products), **date and time of collection.**
8. Use **NEW** whirl-pak® bag for each sample.
9. Fill out **ONE** sample tracking form ("*Listeria* Testing in Food Processing Establishments") for **each facility** (this is a multi-sample form; all samples, environmental and food, can be listed onto this single form). Ensure that **ALL** the fields are filled-out and the adequate information is provided.
10. Place all the samples from a facility together in a plastic bag with the corresponding sample tracking form.
11. Use separate bags for each facility.
12. Place the bag containing the food samples into a clean and sanitized cooler with ice packs.
13. Keep the samples at refrigeration temperatures (i.e. 0 to 4°C) and bring them or ship the cooler to the laboratory as soon as possible or **within 24 hours of sample collection**.
14. Do **NOT** freeze the samples.

Table 1. List of requested sampling sites in different food processing environments.

Facilities		Type of surface		
		Non-food Contact	**Close to Food**	**Food Contact**
Dairy	**Mandatory**	Drain in RTE* area	Wall close/adjacent to food handling surfaces	Worktable
	Choose **ONE**	- Legs of a cart in RTE area or - Legs of a conveyor in RTE area	- Legs of a slicer in RTE area or - Legs of a packaging table in RTE area	- RTE area cheese rack or - RTE cheese slicer or - Inside the milk filler
Meat	**Mandatory**	Drain in RTE area	Wall close/adjacent to food handling surfaces	Meat slicer
	Choose **ONE**	- Legs of a cart in RTE area or - Legs of a conveyor in RTE area	- Legs of a slicer in RTE area or - Legs of a packaging table in RTE area	- Worktable in RTE area or - RTE meat rack
Fish	**Mandatory**	Drain in RTE area	Wall close/adjacent to food handling surfaces	Worktable
	Choose **ONE**	- Legs of a cart in RTE area or - Legs of a conveyor in RTE area	- Legs of a packaging table in RTE area or - Legs of a slicer in RTE area	- RTE fish slicer (post smoked) - Rack or shelf that holds RTE fish products
Other	**Mandatory**	Drain in RTE area	Wall close/adjacent to food handling surfaces	Worktable
	Choose **ONE**	- Legs of a cart in RTE area or - Legs of a RTE area conveyor - Light switch in RTE area	- Legs of a packaging table in RTE area or - Legs of a slicer in RTE area or - Sides of food processing machines in RTE area	- Cutting utensils - Rack or shelf that holds food in RTE area - Packaging table surface in RTE area

*RTE area: Ready-to-eat product handling area.

This page intentionally left blank.

APPENDIX 2

Sample Tracking Form

This page intentionally left blank.

SAMPLE TRACKING FORM

Listeria Testing in Food Processing Facilities

CALL [redacted] JUST BEFORE SHIPPING THE SAMPLES TO CONFIRM THE SHIPPMENT DETAILS! Please see reverse for instructions on sample collection.

[redacted]

Facility Name:	**City:**	**Sampling Date:**
Type of food processed: *Please Select One* ❒ **Dairy** ❒ **Meat** ❒ **Fish** ❒ **Other** _____ Specify		**Sampling Time:**
Collected by:		**Lab Use**

Environmental Samples			**Food Samples**	
Type of surface *Please select one for each sample*	**Sampling site**		***Provide enough details to differentiate similar foods e.g. type of food, processing, ingredients etc.***	
❒ Non-food contact ❒ Close to food ❒ Food contact	1	1	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY
❒ Non-food contact ❒ Close to food ❒ Food contact	2	2	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY
❒ Non-food contact ❒ Close to food ❒ Food contact	3	3	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY
❒ Non-food contact ❒ Close to food ❒ Food contact	4	4	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY
❒ Non-food contact ❒ Close to food ❒ Food contact	5	5	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY
❒ Non-food contact ❒ Close to food ❒ Food contact	6	6	**Product Name** **Brand Name** **Description**	**Lot No.** Provide one: ❒ Best Before Date ❒ Batch Date _____ YEAR / MO NTH / DAY

CONTACT JOVANA KOVACEVIC AT LEAST A WEEK PRIOR TO SAMPLE COLLECTION FOR CONCERNS REGARDING THE SAMPLE COLLECTION SCHEDULE!

Comments

This page intentionally left blank.

APPENDIX 3

Control Measures for Samples Positive for *Listeria* species

This page intentionally left blank.

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Control Measures for Samples Positive for *Listeria* species

For

Occurrence and distribution of *Listeria* species in facilities producing ready-to-eat foods under provincial inspection authority in British Columbia
PROJECT

Prepared by:

Food Protection Services
Issue Date: August 31, 2009
604.707.2440

Food Protection Services, Environmental Health Services Div
Main Floor 655 12th Ave W, Vancouver BC V5Z 4R4
www.bccdc.ca
Tel 604.707.2440 | Fax 604.707.2441

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Scope

The "Control measures for samples positive for *Listeria* spp." document is based on the Health Canada's Policy on *Listeria monocytogenes* in ready-to-eat foods and Canadian Food Inspection Agency's "Meat hygiene manual of procedures". The purpose of this document is to provide guidance for the food industry and the provincial health authorities regarding the compliance actions and control measures when faced with samples positive for *Listeria* species, specifically *L. monocytogenes* (*L. m.)*, as part of the project that is assessing the occurrence and distribution of *Listeria* in food processing facilities in British Columbia.

This document describes different approaches for follow up actions when samples are positive for *Listeria* species and *L. monocytogenes.* It outlines the plan for communication of positive *Listeria* results to the provincial health authorities and industry. It also looks at different types of samples (i.e. food and environmental swabs) and implications that positive results may have for each type.

Although this project is a prevalence survey, *Listeria* found in ready-to-eat (RTE) foods may have severe consequences to vulnerable populations. In light of the recent Weatherill report (July 2009) positive results detected in either RTE foods or on food contact surfaces will be communicated for further follow-up and public health actions.

2. Review of *Listeria* Testing Procedure for This Study

2.1 Summary of the testing procedure

A conventional culture methods (Health Canada's MFHPB – 30 for both environmental and food samples; with slight modifications in regards to the plating intervals, and MFLP-74 enumeration method for food samples, with minor modification in dilution broth) is being used to detect *Listeria* spp. in the samples submitted for this project. The MFHPB-30 culture method is based on four successive stages to detect *Listeria* spp., and where possible further identify *L. monocytogenes*. These steps involve: 1) primary enrichment (*Listeria* Enrichment Broth), 2) secondary enrichment (Modified Fraser Broth), 3) plating and identification (Oxford and PALCAM media) and 4) confirmation of presumptive positive samples. The method is very sensitive; however, it may require seven to 10 days to complete for a single sample. An outline of the processing steps and the expected time-frames are provided in Table 1.

Table 1. The outline of procedure for processing of environmental and food samples for detection of *Listeria* spp. and *L. monocytogenes*, with respect to time required to obtain final results.

Test Procedure	Time Required to Final Result
Direct plating (food samples only)	2 days
Primary Enrichment	2 days
Secondary Enrichment	2 days
Plating out and identification	2 days
Confirmation	2 to 4 days

2.2 Background information on *Listeria*:

The genus *Listeria* is comprised of at least six species. These include *L. monocytogenes, L. ivanovii, L. welshimeri, L. seeligeri, L. innocua,* and *L. grayi* (Rocourt and Buchrieser, 2007). Generally, two species are associated with illness known as listeriosis – *L. monocytogenes* in humans and *L. ivanovii* in other mammals. Once ingested, *L. monocytogenes* is known to penetrate the intestinal lining and multiply in the host, with capabilities of crossing the placental barrier and capillary epithelium, which may lead to abortion, septicemia and meningoencephalitis (Painter and Slutsker, 2007). In healthy individuals, infection can be mild and self limiting with flu-like symptoms, however, for immunocompromised, elderly and very young it may result in severe health complications. Generally, the incidence of listeriosis is low compared to other food-borne illnesses, but the associated mortality is much higher at around 30% (Wing and Gregory, 2000).

3. Proposed guidance for environmental samples

There are three types of environmental samples being collected in this study:

1. Non-food Contact Surfaces (NFCS)
2. Close-to food Contact Surfaces (CTF)
3. Food Contact Surfaces (FCS)

Proposed actions for positive results are escalating dependent on the type of sample and the results (either *Listeria* spp. or *Listeria monocytogenes*). Refer to Figure 1.

3.1 Environmental samples positive for *Listeria* spp.

- In each case the laboratory will contact the Health Authority (HA) unless stated otherwise. The laboratory will perform the test to discriminate between species within 48 hours.
  - ⇒ If a NFCS is positive no action will be taken, the HA will not be contacted.
  - ⇒ If CTF, or FCS is positive the HA will be informed verbally of the result. The Environmental Health Officer (EHO) or Food Safety Specialist (FSS) are recommended to request the Premise Operator (PO) clean and sanitize their premise in the case of CTF positive, and further re-test for *Listeria* within five days of cleaning if a FCS is positive at the cost of the operator.

3.2 Environmental samples positive for *Listeria monocytogenes*

- In each case the laboratory will contact the HA unless stated otherwise.
  - ⇒ If a NFCS is positive the EHO/FSS are recommended to request the PO clean and sanitize their premise.
  - ⇒ If a CTF is positive the EHO/FSS are recommended to further request the PO re-test for *Listeria* within five days of cleaning at the cost of the operator.
  - ⇒ The EHO/FSS are recommended to review the Food Safety Plan and Sanitation Plan with the PO, and further, to hold the food prepared by the PO on the date the sample was collected. The PO should conduct testing at their cost to verify the food is not contaminated.

Note: EHO/FSS are reminded that results for food are given approximately two weeks after collection, and there may be very little food left at the premise if it was a short-shelf life product.

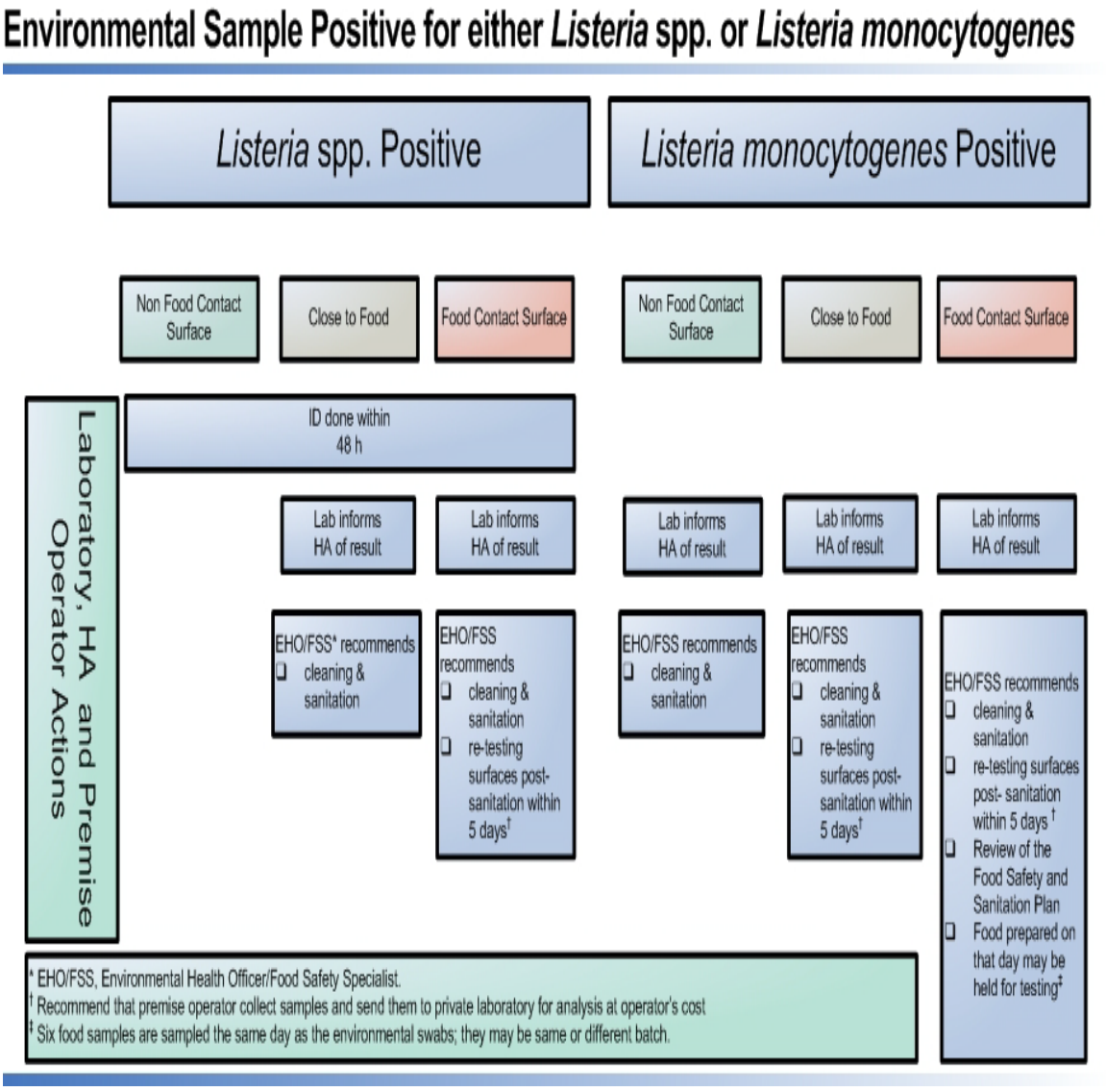


Figure 1. The recommended actions for laboratory, Health Authorities and premise operators when environmental samples positive for either *Listeria* spp. or *L. monocytogenes* are encountered.

4. Proposed guidance for food samples

The Health Canada "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods" does not have a zero tolerance for *Listeria monocytogenes* in all food. The level of risk is assessed by the food type, whether the food is potentially hazardous, how long it is stored, whether the industry producing food has acceptable Good Manufacturing Practices (GMPs) and finally, to whom the food is destined to be served to.

Food is placed into three different categories, depending on the Health Risk. The highest risk foods, placed in Category 1 include:

1. Soft cheese
2. Liver pate
3. Unacidified jellied pork tongue
4. Hot dogs/wieners
5. Cold smoked trout/salmon
6. Processed deli meats

The CFIA Meat Hygiene guidelines on how to follow-up unsatisfactory results were also considered in these recommendations. Actions described include: enhanced cleaning and sanitation, follow-up testing for *L. monocytogenes* within five days of cleaning/sanitation, review of the Food Safety Plan (CFIA refers to this as HACCP) and Sanitation Plan, Health Risk Assessment, and hold and test procedures for products.

The proposed control actions in this document include most aspects of the two sources referenced in the paragraphs above; however, the hold and test procedures were not deemed suitable for the purpose of this project.

4.1 RTE food samples positive for *Listeria* spp.

See Figure 2.

- In each case the laboratory will contact the HA unless stated otherwise.

The laboratory will perform the test to discriminate between species within 48 hours.

⇒ If a food sample is positive the EHO/FSS are recommended to "order" the PO clean and sanitize their premise and re-test for *Listeria* within five days of cleaning at the cost of the PO.

⇒ The EHO/FSS are recommended to review the Food Safety Plan (FSP) and Sanitation Plan (SP) with the PO, and further,

⇒ The EHO/FSS are recommended to hold the food prepared by the PO on the date the sample was collected.

⇒ An assessment of the food should be conducted by the EHO/FSS to verify whether the food is potentially hazardous for the growth of *L. monocytogenes.* The assessment should also include an assessment of the GMPs at the premise. Poor GMPs increase the food risk.

⇒ The laboratory will assess the pH and water activity (a_w) of the food positive for *Listeria* spp. within 24 h (given that the adequate amount of food was provided), OR, ask that the EHO/FSS obtain a second sample of the same batch or same type of food for testing.

Note: EHO/FSS are advised to send in at least 200g of sample to allow for the possibility of pH/a_w testing.

⇒ Post assessment of the food – if the food is Potentially Hazardous Food (PHF), the premises must destroy the batch, re-work the batch or conduct testing at their cost to verify the food is not contaminated.

⇒ Post assessment of the food – if the food is not PHF, the sale is permitted and the lot may be released at the discretion of the HA.

⇒ The HA/EHO/FSS will inform the premise of the final decision.

4.2 RTE food samples positive for *L. monocytogenes*

Refer to Figure 3.

- In each case the laboratory will contact the HA unless stated otherwise.
  - ⇒ If a food sample is *L. monocytogenes* positive, in addition to the HA, the laboratory will contact
    (1) the Director, Food Protection Services, BCCDC *and*
    (2) Epidemiology, BCCDC *and*
    (3) Other requesting agencies
  - ⇒ If a food sample is positive for *L. monocytogenes* the EHO/FSS are recommended to "order" the PO clean and sanitize their premise and re-test for *L. monocytogenes* within five days of cleaning at the cost of the PO.
  - ⇒ The EHO/FSS are recommended to review the Food Safety Plan and Sanitation Plan with the PO.
  - ⇒ The EHO/FSS are recommended to order a hold on the food prepared by the PO on the date the sample was collected and further, to hold the food from the previous batch and subsequent batch if available.
  - ⇒ If the food is a Category 1 food (as defined in the list by Health Canada) the HA is advised to issue a regional recall of the contaminated batch. This batch must be either destroyed or re-worked. If the PO chooses to re-work the product they must re-test the product at their own cost to verify the food is not contaminated.
  - ⇒ An assessment of the food should be conducted by the EHO/FSS to verify whether the food supports the growth of *L. monocytogenes*. The assessment should also include an assessment of the GMPs at the premise. Poor GMPs increase the food risk.
  - ⇒ The laboratory will assess the pH/a_w of the leftover food that was positive for *L. monocytogenes*, within 24 h (given that the adequate amount of food was provided), OR, ask that the EHO/FSS obtain a second sample of the same batch or same type of food for testing.
    Note: EHO/FSS are advised to send in at least 200g of sample to allow for the possibility of pH/a_w testing.

⇒ If the food does meet the PHF definition, *and*
If the food is refrigerated >10 days, but is not a Category 1 food

The HA is advised to issue a regional recall of the contaminated batch. This batch must be either destroyed or re-worked. If the PO chooses to re-work the product they must re-test the product at their own cost to verify the food is not contaminated.

Food positive for *L. monocytogenes* may only be sold if the following criteria are met.

⇒ IF the food does not meet the criteria of a PHF, *and*,
IF the food is not refrigerated for >10 days, *and*
IF the food has a *L. monocytogenes* count of ≤100 CFU/g, *and*
IF the food is not targeted for sale or distribution to vulnerable populations

The HA may approve sale of the food positive for *L. monocytogenes* at their discretion, otherwise the food should be either destroyed or re-worked.

⇒ The HA/EHO/FSS will inform the premise of the final decision

4.3 Raw food samples positive for *Listeria* spp. or *L. monocytogenes*

⇒ If the raw food sample is RTE (e.g. sashimi), actions, reporting and recommendations as described above (i.e. Sections 4.1. and 4.2) will be followed.

⇒ If the raw food sample is meant to be cooked or further processed, no reporting or follow-up actions are recommended.

5. Reporting and Communications

5.1 Reporting

Since this is a prevalence study, **no written results will be issued for any samples.** In addition, negative results will not be reported. However, because detection of *Listeria* in food can have serious public health implications, positive results will be reported by telephone to agencies directly involved, and other agencies if necessary. These results will only be reported verbally. Persons submitting samples are reminded that they may not receive results for up to two weeks following the sample submission. The laboratory will keep a record of the calls (Figure 4; Figure 5).

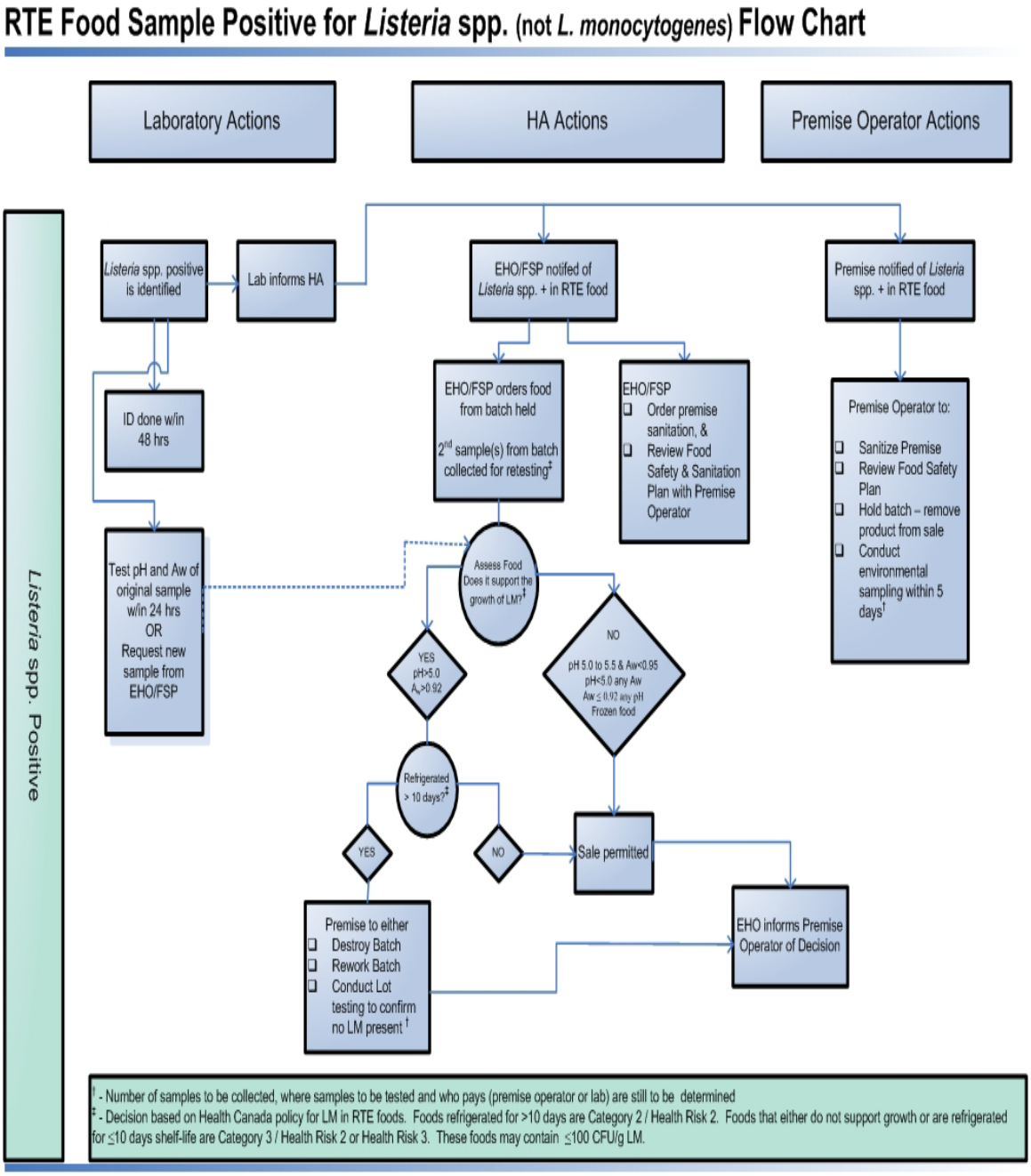


Figure 2. The recommended actions for laboratory, Health Authorities and premise operators when ready-to-eat (RTE) food samples positive for non-pathogenic *Listeria* spp. are encountered (i.e. excludes samples positive for *L. monocytogenes*).

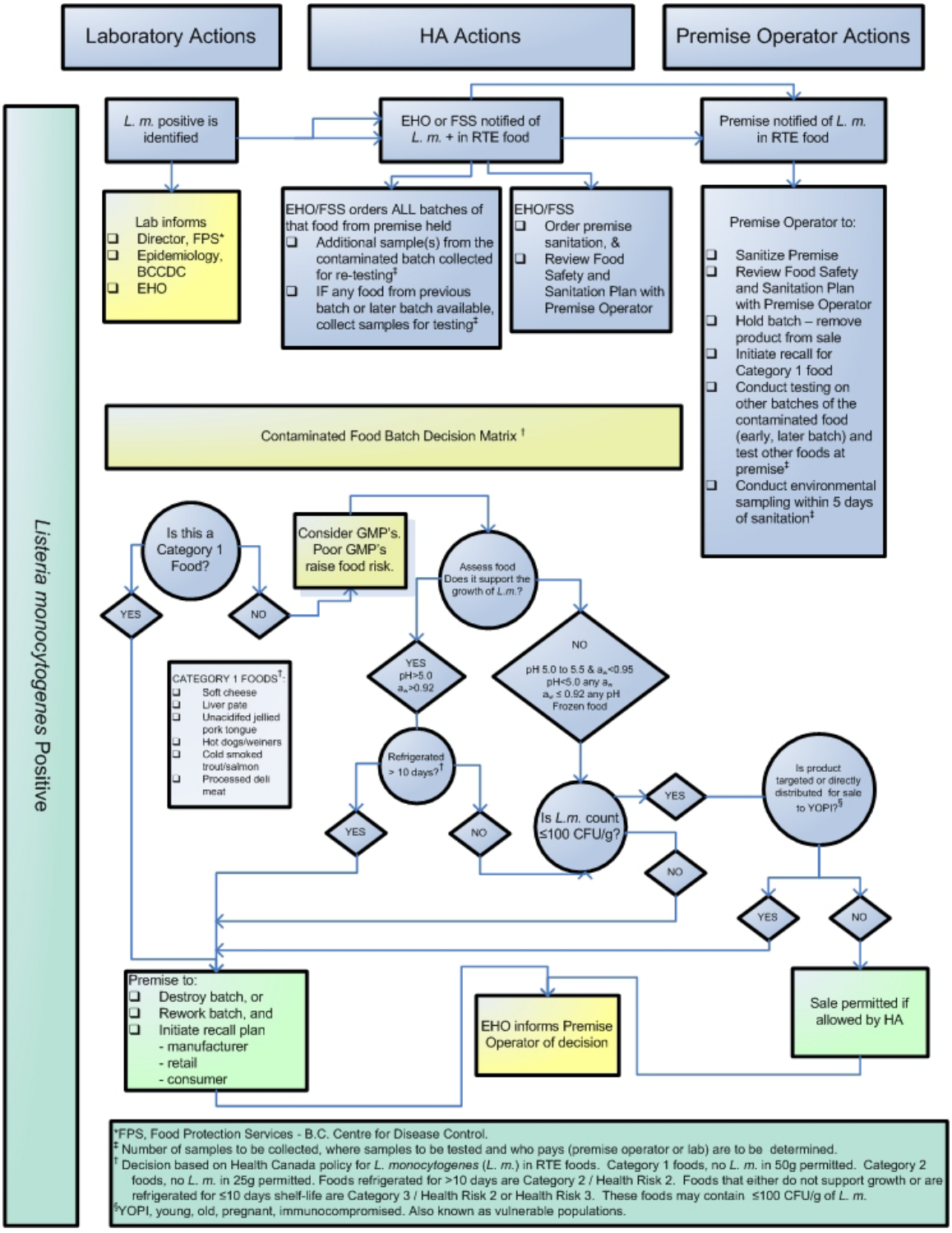


Figure 3. The recommended actions for laboratory, Health Authorities and premise operators when ready-to-eat (RTE) food samples positive for *L. monocytogenes* are encountered.

5.2 Communications

- **Food Protection Services, BC Centre for Disease Control**

The "laboratory" for this project is defined as Food Protection Services, BCCDC. The lead investigator is Jovana Kovacevic, who is currently employed as a Food Safety Specialist by Food Protection Services, BCCDC. She is conducting the laboratory investigation with the permission of PHSA (Provincial Health Services Authority) Laboratories. General inquiries about the project should be directed to Ms. Kovacevic.
Other Food Safety Specialists will be assisting by phoning results and on demand.

All BCCDC phone numbers have changed and are listed below for reference.

Jovana Kovacevic	FPS office:	[redacted]
Lynn Wilcott	FPS office:	
Lorraine McIntyre	FPS office:	
Sion Shyng	FPS office:	
Brian Radke	FPS office:	

- **Communications with Health Authorities**

In the event a food product is found positive for *L. monocytogenes*, the HA in control of the premise where the food was manufactured and collected is advised to review the premise distribution list for that food product to discover whether the food product has been distributed to the retail markets in other regional Health Authorities.
If the food product has gone to the retail market (i.e. consumer level) the food product presents a potential risk to the public. At this juncture, the food product in question may or may not be recalled.

The lead HA is recommended to contact the Food Safety Manager EHO and/or Director at all other HAs to inform them about the food product that tested positive, and to inform them that the food product may potentially be recalled pending an assessment.
During this period it is likely that conference calls/meetings will be organized by either the lead HA or Food Protection Services, BCCDC. The lead HA may choose to invite other affected HAs to the conference calls/meetings, or to inform them of decisions after the calls/meetings, at their discretion. Food Protection Services, BCCDC will make themselves available for assistance at the request of the lead HA.

If a decision is made to recall the food, HAs and BCCDC are advised to follow the guidelines in the "*Provincial Food Recall Directory and Reference Manual*" (version November 2006).

Listeria Positive Phoning Checklist

For ☐ *Listeria* spp. and/or ☐ *L. monocytogenes* (*L.m.*)

Date of Lab Result: _____ Date Samples Collected: _____ Processed: _____

I. Facility and Sample Description

Collected by: _____ EHO Tel: _____

Name of Facility: _____ Facility Tel: _____

Facility Address: _____

☐ **ENVIRONMENTAL SAMPLES**

	☐ Non-food Surface	☐ Close-to-food Surface	☐ Food-contact Surface
Lab# ID(s):			
Positive for:	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*

Sample(s) Description: _____

☐ **FOOD SAMPLES**

Lab# ID(s):			
Positive for:	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*	☐ *Listeria* spp. ☐ Presumptive *L. m.* ☐ Confirmed *L. monocytogenes*

Sample(s) Description: _____

II. Phoning/Verbal Notification of Results

☐ **DIRECTOR, FOOD PROTECTION SERVICES** Date/Time Notified:

Name: _____ ☐ *Listeria* spp. _____

Tel/Cell: _____ ☐ *L. monocytogenes* _____

Comments: _____

☐ **HEALTH AUTHORITY** Date/Time Notified:

Name: _____ ☐ *Listeria* spp. _____

Tel/Cell: _____ ☐ *L. monocytogenes* _____

Comments: _____

Date/Time Notified:

Name: _____ ☐ *Listeria* spp. _____

Tel/Cell: _____ ☐ *L. monocytogenes* _____

Comments: _____

☐ **EPIDEMIOLOGY, BCCDC** Date/Time Notified:

Name: _____ ☐ *Listeria* spp. _____

Tel/Cell: _____ ☐ *L. monocytogenes* _____

Comments: _____

Figure 4. The record of verbal notification for environmental and food samples positive for *Listeria* spp. used by the laboratory and Food Protection Services to notify Health Authorities and Epidemiology Division, BCCDC.

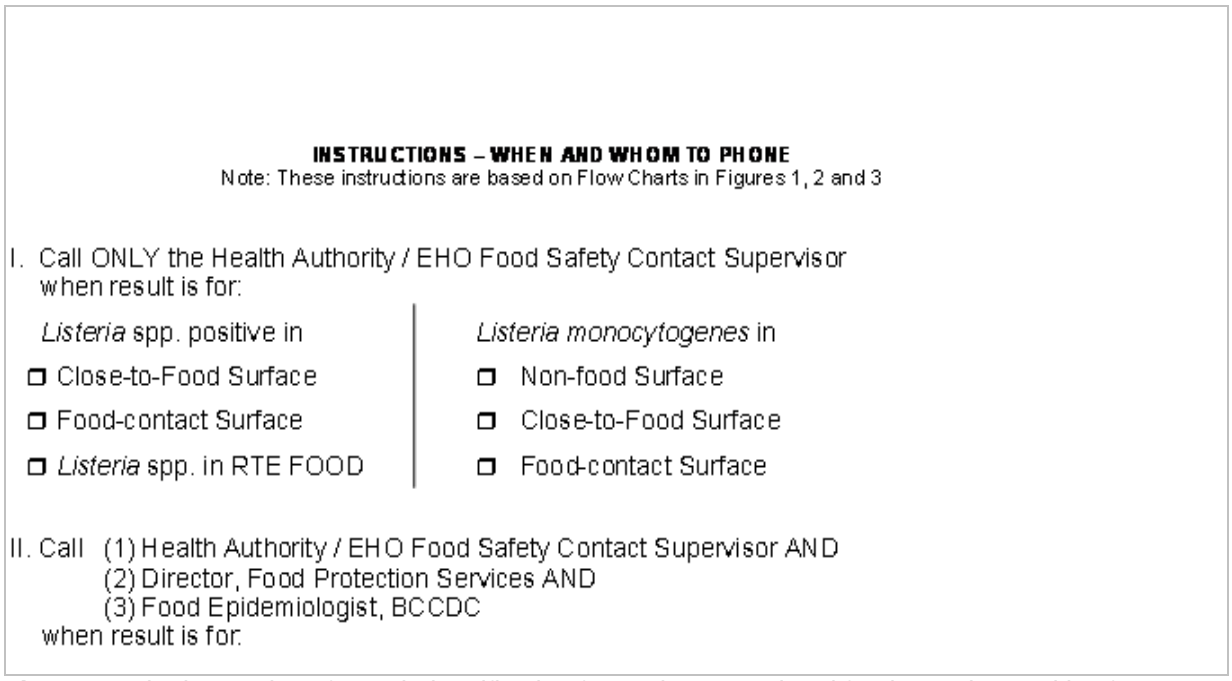


Figure 5. The instructions for verbal notification for environmental and food samples positive for *Listeria* spp. used by the laboratory and Food Protection Services to notify Health Authorities and Epidemiology Division, BCCDC.

6. Considerations for the review of information in regards to food subjected to a potential recall – a health risk event:

- Quantity of the contaminated batch
  Note: Positive food is from a single batch, and this batch is designated as the contaminated batch.
- How many other batches were made in that day, and quantity of products
- How many other batches were made in that week (after the contaminated batch), and quantity of products
- When was the production halted
- Considering the distribution information, define the distribution categories:
  - sold directly to consumer, general public
  - sold to retail or restaurant
  - sold directly to institutions (e.g., hospitals)
  - sold to secondary distributor

  Note: this information is helpful to define what types of recalls may be required
- Considering the distribution information, is the product sold outside of the regional HA?
- What is the shelf-life of the food?
- What is the storage temperature for the food?
  - Is food refrigerated, frozen, or at room temperature?
  - Is food frozen and then thawed before sale?

(e.g., potential to have longer shelf-life)

- How is the product displayed, labeled and sold to the consumer?
  - At the retail level, is there information given to the consumer that would identify the product as being from a specific processor?
  - Does the food come in packaging that informs the consumer of the expiry date or shelf-life?
  - Does the food come in packaging that informs the consumer of the storage temperature for the food?
  - Is the food displayed refrigerated, on ice and/or in bulk? Is it labeled / unlabeled?
- Is the product transformed or used as an ingredient to make any other food types?

Additional items to consider

- Based on the inspector observations does the processing plant have good or poor GMPs?
- Were the environmental sampling results satisfactory?
- Is the processing plant following their Food Safety Plan and Sanitation Plan?

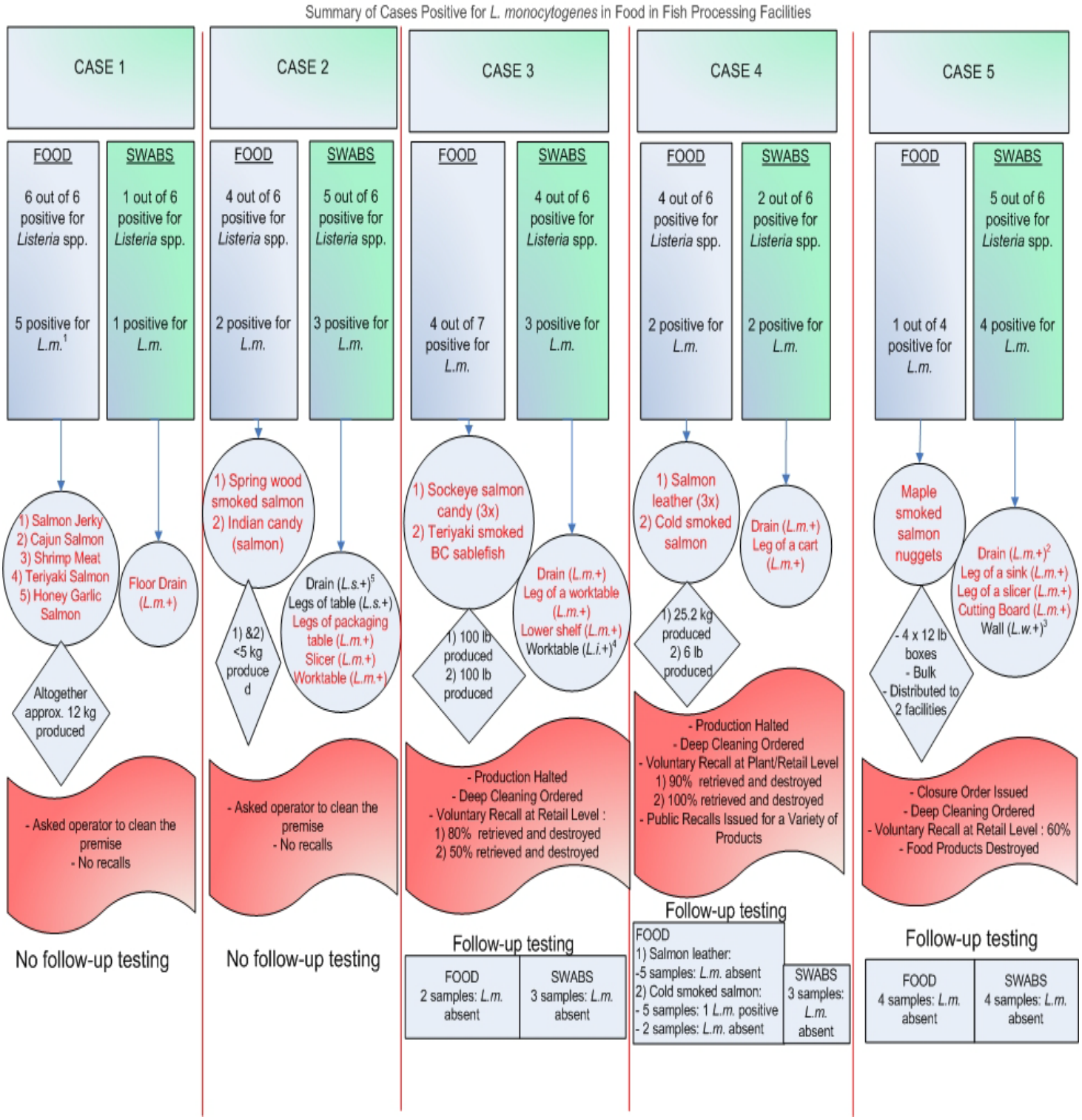
7. References

1. Anonymous, 1996. Microbiology of food and animal feeding stuffs- Horizontal method of the detection and enumeration of *Listeria monocytogenes*- Part 1: Detection method. ISO11290-1., p. 1-16. *In*, International Organization for Standardization, Geneva, Switzerland.
2. BC Centre for Disease Control, 2006. Provincial Food Recall Directory and Reference Manual. Revised November, 2006.
3. Canadian Food Inspection Agency, 2009. Meat Hygiene Directive 2009-1. Control measures for Listeria in ready-to-eat (RTE) meat products. Chapter 5. Accessed from http://www.inspection.gc.ca/english/fssa/meavia/man/direct/2009/direct1e.shtml
4. Farber, J. M. and D. Warburton, 1998. Isolation of *Listeria monocytogenes* from all food and environmental samples. MFHPB-30. Accessed from http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb30-01-eng.php
5. Food Directorate, 2004. Policy of *Listeria monocytogenes* in Ready-to-Eat Foods. Health Products and Food Branch, Health Canada. Accessed from http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_politique_toc-eng.php
6. Pagotto, F., E. Daley and J. M. Farber. 2002. Enumeration of *Listeria monocytogenes* in foods. MFLP-74, p.1-5. *In* D. Warburton (ed.) Compendium of Analytical Methods. Health Canada Methods of Microbiological Analysis of Foods, vol. 2. Polyscience Publications Inc., Montreal.
7. Painter, J., and L. Slutsker. 2007. Listeriosis in humans, p.85-109. *In* E.T. Ryser and E.H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 3rd Ed., CRC Press, Boca Raton, FL.
8. Rocourt, J., and C. Buchrieser. 2007. The Genus *Listeria* and *Listeria monocytogenes*: Phylogenetic position, taxonomy, and identification, p.1-20. *In* E.T. Ryser and E.H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 3rd Ed., CRC Press, Boca Raton, FL.
9. Weatherill, 2009. Report of the Independent Investigator into the 2008 Listeriosis Outbreak. Accessed from http://www.listeriosis-listeriose.investigation-enquete.gc.ca/lirs_rpt_e.pdf
10. Wing, E.J. and Gregory, S. H., 2000. An updated model of cell-mediated immunity – listeriosis: clinical and research aspects. *Allergy Asthma Proc*. 21: 209-214.

APPENDIX 4

Summary of Cases Positive for *L. monocytogenes* in Ready-to-eat Products and Processing Facilities

This page intentionally left blank.



[1]*L.m.*, *Listeria monocytogenes*; [2]*L.m.*+, *L. monocytogenes* positive; [3]*L.s.*+, *L. seeligeri* positive; [4]*L.i.*+, *L. innocua* positive; [5]*L.w.*+, *L. welshimeri* positive.

This page intentionally left blank.