Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2011-2012 Targeted Surveys

Targeted Survey Investigating Bacterial Pathogens and Generic *E. coli* in Leafy Vegetables

RDIMS#4048832

Canada

Table of Contents

Executive Summary

The Food Safety Action Plan (FSAP) aims to modernize and strengthen Canada's food safety system in order to better protect Canadians from unsafe food and ultimately reduce the occurrence of foodborne illness.

In recent years, leafy vegetables have been reported to be associated with numerous outbreaks of foodborne illness worldwide. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has ranked leafy vegetables as the highest priority of concern in terms of microbiological hazards among fresh fruits and vegetables. Leafy vegetables can become contaminated with various foodborne pathogens during production, harvest, post-harvest handling, processing, packaging and distribution. Due to their leafy nature, these vegetables are more easily contaminated than others. As they are often consumed raw, the presence of pathogens creates a potential risk for foodborne illness. The bacterial pathogens *Escherichia coli* (*E. coli*) O157:H7 and *Salmonella* have accounted for the majority of the outbreaks associated with leafy vegetables. *Shigella* and *Campylobacter* have also been implicated in leafy vegetables associated outbreaks. In addition, *Listeria monocytogenes* (*L. monocytogenes*) has been identified as a food safety concern in ready-to-eat (RTE) foods including fresh-cut RTE leafy vegetables due to its wide distribution in the environment and its ability to grow under refrigeration temperatures.

Considering the factors mentioned above and their relevance to Canadians, leafy vegetables have been selected as one of the priority commodity groups of fresh fruits and vegetables for enhanced surveillance under the FSAP. Over the course of a five-year baseline study (2008/09 - 2012/13), approximately 10,000 leafy vegetable samples were collected from Canadian retail locations and tested for various pathogens of concern. The main objectives of the 2011/12 survey were to generate baseline surveillance data on bacterial pathogens *E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, and *Listeria monocytogenes*, as well as on an indicator of fecal contamination, generic *E. coli*, for a variety of leafy vegetables available in the Canadian market.

A total of 1537 imported and domestic samples, including 320 whole leafy vegetable samples and 653 fresh-cut leafy vegetable samples, were collected and tested for the targeted bacteria. Bacterial pathogens *E. coli* O157, S*almonella*, *Shigella*, and *Campylobacter* were not detected in any of the samples. One of the fresh-cut samples (0.2%) was found to have an elevated, yet marginally acceptable, level of generic *E. coli*. Generic *E. coli* is an indicator used by the Canadian Food Inspection Agency (CFIA) to assess general sanitation and hygiene practices throughout the production chain. Two of the fresh-cut samples (0.3%) were assessed as unsatisfactory due to the presence of

L. monocytogenes. Since the products were already passed their stated shelf-life when the results were confirmed, there were no related products recalled. The CFIA conducted appropriate follow-up activities for the contaminated products, including a joint inspection with the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) at one of the processing facility. No reported illnesses were found to be associated with the contaminated products during this survey. These results suggest that the vast majority of fresh leafy vegetables in the Canadian market sampled during this survey were produced under Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs). Sporadically, *L. monocytogenes* contamination in fresh-cut leafy vegetables can occur.

The CFIA regulates and provides oversight to the industry, works with provinces and territories, and promotes safe handling of foods throughout the food production chain. However, it is important to note that the food industry and retail sectors in Canada are ultimately responsible for the food they produce and sell, while individual consumers are responsible for the safe handling of the food they have in their possession. In addition, general advice for the consumer on the safe handling of foods is widely available. The CFIA will continue its surveillance activities and inform stakeholders of its findings.

1 Introduction

1.1 Food Safety Action Plan

In 2007, the Canadian government launched a five-year initiative in response to a growing number of product recalls and concerns about food safety. This initiative, called the Food and Consumer Safety Action Plan (FCSAP) (1), aims to modernize and strengthen Canada's safety system for food, health and consumer products. The FCSAP initiative unites multiple partners in ensuring safe food for Canadians.

The Canadian Food Inspection Agency's (CFIA) Food Safety Action Plan (2) is one element of the government's broader FCSAP initiative. The goal of the FSAP is to identify risks in the food supply, limit the possibility of occurrence of these risks, improve import and domestic food controls, and identify food importers and manufacturers.

Within the FSAP, there are 12 main areas of activity, one of which is risk mapping and baseline surveillance. The main objective of this area is to better identify, assess and prioritize potential food safety hazards through risk mapping, information gathering and analysis of foods in the Canadian marketplace. Targeted surveys are one tool used to test for the presence and level of particular hazards in specific foods.

1.2 Targeted Surveys

Targeted surveys are used to gather information regarding the potential occurrence of hazards in food commodities. The microbiological targeted surveys aim to establish baseline data on priority and/or emerging microbiological hazards in targeted commodities, primarily fresh fruits and vegetables and imported food ingredients. A statistically significant number of samples were collected over five years to allow for seasonal and/or production variations. This work differs from regular CFIA microbiological monitoring activities which test samples of a broad range of commodities for multiple hazards and are aimed to determine the compliance of defined lots with established microbial standards or guidelines for regulatory purposes.

To identify food-hazard combinations of greatest potential health risk for the targeted surveys, the CFIA uses a combination of scientific literature, documented outbreaks of foodborne illness, and/or information gathered from the Food Safety Science Committee (FSSC), a group of Canadian federal, provincial and territorial subject matter experts in the area of food safety (3).

This survey (2011/12) represents part of the collection of over 10,000 leafy green vegetable samples over five years (2008/09 – 2012/13) of microbiological targeted

surveys and was designed to gather baseline information on the occurrence of bacterial pathogens of concern as well as the presence and levels of generic *E. coli* in leafy vegetables available to Canadians at retail.

1.3 Codes of Practice, Acts, and Regulations

International food safety standards, codes of practice, and guidelines relating to food, food production, and food safety are developed under the joint Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) Codex Alimentarius Commission. Producers of fresh fruits and vegetables are encouraged to follow the international codes of practice. Of relevance for this survey are the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CAC/RCP 53-2003) (4) and the *Recommended International Code of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969) (5). These codes address Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) which, when applied, control and reduce the potential for contamination with microbial, chemical, and physical hazards at all stages of the production of fresh fruits and vegetables from primary production to packaging.

Fresh fruits and vegetables available in the Canadian market must comply with the *Food and Drugs Act* (FDA) (6) and the *Food and Drug Regulations* (FDR) (7), which prescribe certain restrictions on the production, importation, sale, composition and content of foods and food products. Section 4(1)a of the FDA prohibits the sale of food contaminated with foodborne pathogens, while sections 4(1)e and 7 prohibit the sale of unsafe food and food produced under unsanitary conditions.

Fresh fruits and vegetables that are imported in Canada or domestically produced and marketed inter-provincially must also comply with safety requirements of the *Fresh Fruit and Vegetable Regulations* (8) under the *Canada Agricultural Products Act* (9). These regulations are intended to ensure that fresh fruits and vegetables sold to consumers are safe, wholesome and properly graded, packaged and labeled.

The *Fresh Fruit and Vegetable Regulations* and the food-related portions of the FDA and FDR are enforced by the CFIA.

The FSAP targeted surveys are primarily conducted for surveillance and not for regulatory compliance verification purposes. However, results indicating a potential risk to public health for any samples tested under this survey will trigger food safety investigations, including activities such as follow-up sampling, inspections of facilities, and health risk assessments. Depending on the findings, a recall of the affected product may be warranted.

2 Survey on Fresh Leafy Vegetables

2.1 Rationale

Leafy vegetables have been reported to be responsible for numerous outbreaks of foodborne illnesses worldwide. From 1998 to 2011, 62 foodborne disease outbreaks associated with leafy vegetables contaminated with bacterial pathogens were reported worldwide, with most of the reported cases occurring in North America including several cases in Canada (10) (11) (Appendix B & C).

Production practices can affect the microbial load of leafy vegetables. For example, the use of improperly composted animal manure has led to concerns about the potential contamination of produce with human pathogens. Since organic productions are more reliant on the use of manure to fertilize fields, it has been suggested, while not proven yet, that organic produce may face higher levels of microbial contamination. In contrast, hydroponically grown vegetables (e.g., head lettuces) may face a lower likelihood of being contaminated with pathogens since these vegetables are not in contact with soil and soil amendments and are not exposed to floods and animals. However, one study suggests that there is still a potential risk for hydroponic leafy vegetables to harbor a pathogen from fecal contamination, as a low percentage of the hydroponic leafy vegetable samples (14%, 16/114) tested during that study was found to contain generic *E. coli* (12).

Processing (e.g., cutting, shredding, and packaging) and storage of fresh-cut vegetables may also provide further opportunities for cross-contamination and potential for growth of bacterial pathogens. For example, cutting releases fluid from the vegetables, which promotes the growth of bacteria (13). Furthermore, inappropriate temperatures during preparation, distribution and/or storage can also encourage the growth of bacteria on Ready-to-Eat (RTE) fresh-cut leafy vegetables (14) (15).

Leafy vegetables were identified as a level one (highest) priority of concern in terms of microbiological hazards among fresh fruits and vegetables during a joint FAO/WHO Expert Meeting in 2007 (16). This was based on multiple factors, such as historical outbreaks, potential for contamination, and other evidence (e.g., exposure levels, outbreaks with high number of illnesses, etc).

Based on the above information and the Food Safety Science Committee's recommendations (3), fresh leafy vegetables have been selected for targeted surveillance under the FSAP for five years (2008/09 to 2012/13). The overall objective of this five-year study is to gather baseline information on the occurrence of various pathogens

(bacteria, viruses and parasites) of concern in leafy vegetables available to Canadians at retail.

This targeted survey (2011/12) is part of the information collection with a focus on investigating the presence and distribution of bacterial pathogens, as well as the presence, distribution, and levels of generic *E. coli* (as an indicator of fecal contamination) in imported and domestic, conventional and/or organically produced leafy vegetables.

2.2 Targeted Microorganisms

2.2.1 Bacterial Pathogens of Concern

Bacterial pathogens *Salmonella* and *E. coli* O157 are found naturally in the intestines of animals, such as poultry and cattle, respectively (17). Most outbreaks associated with these bacterial pathogens are linked to the consumption of contaminated food of animal origin (e.g., chicken and beef burger). However, fresh fruits and vegetables have emerged as significant sources of these bacterial pathogens related illnesses in the last decade (10). Fruits and vegetables can become contaminated with these bacterial pathogens in the field by improperly composted manure, contaminated water, wildlife feces, and/or poor hygienic practices of the farm workers (18).

Humans are the only host of the bacterial pathogen *Shigella*. Food contaminated by infected food handlers with poor personal hygiene, and water contaminated with human feces are the most common causes of shigellosis. Shigellosis illnesses have been known to be associated with consumption of contaminated fruits, vegetables, shellfish, and chicken (17).

Similarly to *Salmonella* and *E. coli* O157, bacterial pathogen *Campylobacter* is also found naturally in intestines of most food-producing animals, such as chicken, swine, and cattle. *Campylobacter* is one of the leading bacterial causes of foodborne illnesses in the U.S. (19) and Canada (20). Raw poultry and unpasteurized (raw) milk are major sources of contaminated food. However, vegetables were also found, sporadically, to be contaminated with *Campylobacter* (17).

L. monocytogenes is widely distributed in the environment and has been isolated in a wide variety of foods, including raw vegetables. Likely sources of vegetable contamination include soil, contaminated irrigation water or wash water, decaying vegetation, as well as the processing and packaging environment. Compared to other bacterial pathogens, *L. monocytogenes* has an abnormally wide range of growth temperatures (i.e., -0.4 to 45ºC) that includes the typical refrigeration temperature of 4°C (21). Contaminated fresh-cut vegetables that are capable of supporting the limited

growth of the bacteria at refrigeration temperatures have been implicated in a few outbreaks of foodborne listeriosis (21).

2.2.2 Generic *E. coli* as an Indicator of Fecal Contamination

Typically, *E. coli* bacteria that inhabit the large intestines of humans and animals are harmless. Due to their regular presence in stools of humans and animals, the occurrence of *E. coli* in foods indicates direct or indirect contamination with fecal matter. The presence of generic *E. coli* in foods can also indicate potential contamination with pathogenic enteric microorganisms, such as *Salmonella* or *E. coli* O157, that also live in the intestines of infectious humans and animals. It is important to note that the presence of generic *E. coli* in food only implies an increased risk of contamination with pathogenic microorganisms but does not conclusively indicate that these pathogenic organisms are present. High levels of generic *E. coli* in fresh produce sold at retail are an indication that contamination has occurred at some point between primary production and the time of sale.

2.3 Sample Collection

Leafy vegetable samples consisted of arugula, escarole, endive, chicory, varieties of lettuce (e.g., head lettuce, leaf lettuce, and romaine lettuce), spinach, Swiss-chard, watercress, and baby varieties of the above. Leafy vegetables that had been peeled, sliced, chopped or shredded prior to being packaged for sale were categorized as fresh-cut. Head lettuce samples mainly consisted of iceberg lettuce, butter head lettuce and Boston lettuce.

All samples were collected from national chain and local/regional grocery stores, other conventional retail and natural food stores located in various cities across Canada. The number of samples collected in the various regions was based on the relative proportion of the population in the respective regions. Samples were collected during 2011/12 fiscal year (April 1, 2011 to March 31, 2012). Domestic samples were mainly collected during the late summer months in this survey. Imported samples were collected primarily in the fall, winter, and spring months. Samples that were labeled as organic at retail were identified as “organic” in this survey. Other samples were identified as “conventional”.

In this survey, a sample consisted of a single sample unit (e.g., individual consumer-size package(s) from a single lot) with a total weight of at least 200 g. This sampling approach has been used for many retail food surveys (22), (23), (24) and by other federal partners such as the Public Health Agency of Canada (PHAC) under the FoodNet retail surveillance (25).

Collected samples were required to be shipped under conditions that limited the growth of microorganisms during transit. If issues or questions arose about the conditions in which the sample was shipped, the sample was declared unfit for analysis.

2.4 Sample Distribution

As per survey design, three groups of leafy vegetable samples were collected and analyzed for specific combinations of targeted microorganisms (Table 1).

Table 1 Sample Distribution by Targeted Pathogen Group

Objective Group	Targeted Microorganisms	Products Origin	Production Practice	Number of Samples
Group I (whole leafy vegetables)	*E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, and generic *E. coli*	Domestic	Conventional	238
			Organic	82
		Subtotal		***320***
Group II (fresh-cut leafy vegetables)	*E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, and generic *E. coli*	Domestic	Conventional	219
			Organic	42
		Imported	Conventional	57
			Organic	335
		Subtotal		***653***
Group III (head lettuce)	*E. coli* O157, *Salmonella*, *Shigella*, and generic *E. coli*	Domestic	Conventional	294
		Imported	Conventional	266
			Organic	4
		Subtotal		***564***

2.4.1 Sample Distribution by Country of Origin

All domestic samples were grown from various provinces across Canada. The majority of imported samples were from the U.S. (Table 2).

Table 2 Sample Distribution by Country of Origin

Country of Origin	Group I (whole leafy vegetables)	Group II (fresh-cut leafy vegetables)	Group III (head lettuce)
Canada	320 (100%)	261 (40%)	294 (52.1%)
China	0	0	1 (0.2%)
France	0	0	1 (0.2%)
Mexico	0	30 (4.6%)	3 (0.5%)
United States	0	356 (54.5%)	263 (46.6%)
Un-identified	0	6 (0.9%)	2 (0.4%)
Imported-subtotal	*0*	*392 (60%)*	*270 (47.9%)*
Total	**320 (100%)**	**653 (100%)**	**564 (100%)**

2.4.2 Sample Distribution by Product Type

The product types were tabulated for each defined leafy vegetable group (Table 3). A variety of lettuces accounted for approximately 58.1% and 53.7% of the whole (group I) and fresh-cut (group II) leafy vegetable samples, respectively. A majority (94.3%) of the samples were lettuces in the head lettuce group (group III).

Table 3 Product Type in Each Group of Leafy Vegetable Samples

Product Type	Group I (whole leafy vegetables)	Group II (fresh-cut leafy vegetables)	Group III (head lettuce)
Arugula	2	8	0
Chicory	23	3	31
Collard	6	8	1
Dandelion	7	5	0
Kale	22	15	0
Spinach	34	49	0
Spring mix	0	141	0
Spring mix with herbs	0	38	0
Swiss chard	35	20	0
Watercress	0	4	0
Shredded cabbage	0	3	0
Other*	5	8	0
- Boston/butter lettuce	5	4	147
- Iceberg lettuce	0	14	248
- not specified	0	2	90
Subtotal Lettuce - head	*5 (1.5%)*	*20 (3.1%)*	*485 (86.0%)*
- Romaine lettuce	67	158	24
- Red leafy lettuce	49	14	10
- not specified	65	35	13
Subtotal Lettuce - leaf	*181 (56.6%)*	*207 (31.7%)*	*47 (8.3%)*
Lettuce – mix (salad mix)	0	124	0
Total - Lettuce	*186 (58.1%)*	*351 (53.7%)*	*532 (94.3%)*
Total	**320 (100%)**	**653 (100%)**	**564 (100%)**

* Others refer to vegetable types with small number of samples (e.g., one or two samples in total) or when vegetable types were not identified.

2.5 Methods Details

Samples were analysed using the analytical methods as published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods (26) (Appendix D). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of fresh fruits and vegetables, including leafy vegetables. Modified versions of the methods from Health Canada's Compendium were used for *Campylobacter* and *Salmonella*, as indicated in Appendix D.

For the detection of *E. coli* O157:H7/NM, *Salmonella, Shigella*, *Campylobacter,* and *L. monocytogenes,* samples were analyzed by enrichment and confirmed by isolation, purification and identification procedures. The laboratories also had the option of screening enrichment broths by polymerase chain reaction (PCR)-based methods followed by confirmation of presumptive positives. Enumeration was performed on samples that were confirmed positive for *L. monocytogenes.*

The count of generic *E. coli* was obtained using the most probable number (MPN) or direct plating procedure.

2.6 Assessment Guidelines

The assessment criteria used for this survey (Table 4 and Table 5) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* (27) and associated methods published in Health Canada's *Compendium of Analytical Methods* (26), as well as Health Canada's "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (2011)" (21).

Table 4 Assessment Guidelines for Bacterial Pathogens in Leafy Vegetables

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
***E. coli* O157:H7/NM** (MFLP-30 with Supplement 1&2, and MFLP-80)	Absent in 25 g	Present in 25 g
Salmonella* spp.* (MFLP-29 modified and MFHPB-20)	Absent in 25 g	Present in 25 g
Shigella* spp. * (MFLP-26 and MFLP-25)	Absent in 25 g	Present in 25 g
Campylobacter* spp.* (MFLP-46 modified)	Absent in 25 g	Present in 25 g

* *Compendium of Analytical Methods* (26).

**No criteria have been established by Health Canada at this time for these bacterial pathogens in fresh fruits and vegetables. However, in the absence of a specified criteria, presence in foods is considered to be a violation of FDA Section 4(1)a and is therefore assessed by the CFIA as unsatisfactory

Table 5 Assessment Guidelines for Generic *E. coli* in Leafy Vegetables and *L. monocytogenes* in Fresh-cut Leafy Vegetables

Analysis*	Assessment Criteria		
	Satisfactory	**Investigative**	**Unsatisfactory**
Generic *E. coli* (MFHPB-19 & 27)**	≤ 100 /g	100 < x ≤ 1000 /g	> 1000 /g
L. monocytogenes (MFLP-28, MFHPB-30, and MFLP-74)	Absent in 25 g	Detected and ≤ 100 CFU/g	> 100 CFU/g

* Compendium of Analytical Methods (26)

** Concentration unit for MFHPB-19 method: MPN/g, for MFHPB-27 method: CFU/g.

Based on the current regulatory standards and microbiology testing criteria, results of these surveys were assessed as "satisfactory" "unsatisfactory", or "investigative".

Unsatisfactory sample assessments were subject to follow-up actions, such as directed follow-up sampling, establishment inspection, health risk assessment, and/or product action (e.g., product recall).

Samples assessed as investigative for generic *E. coli* in this survey required some form of follow-up activity. For example, further sampling to verify the levels of generic *E. coli* in the samples in question.

Samples assessed as investigative for *L. monocytogenes* (≤ 100 CFU/g) in this survey also required further evaluation. If the product stated shelf-life was ≤ 5 days, the sample was further assessed as satisfactory. If the product stated shelf-life was > 5 days, the sample was further assessed as unsatisfactory.

2.7 Limitations of the Survey

Samples tested during this survey were collected at retail locations across Canada, as opposed to monitoring samples that are picked up at distribution points and warehouses. As such, products sampled at retail could be mixed and originate from different shipments and/or suppliers. Though this represents what the Canadian consumer experiences, this imposes certain limitations with respect to the traceability of the products and the identification of the source of contamination in the case of positive results.

Results obtained for a targeted survey sample are from the analysis of a single sample unit. This sampling and testing strategy generally precludes the extrapolation of the

laboratory result to the whole production lot as it is not statistically representative. This imposes certain limitations in the interpretation of the results to the specific lot in the absence of additional information.

Finally, given the seasonality, as well as the varying channels of commerce, the source of the products can change dramatically from one season to the next. As such, there is an insufficient number of samples in this survey to carry out a detailed analysis of the results based on country of origin. In cases of positive results, unsatisfactory rates between countries are not considered to be statistically comparable.

3 Results

3.1 Whole Leafy Vegetable Samples Analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, and generic *E. coli*

In the whole leafy vegetable group, a total of 320 domestically produced samples, including conventional and organically grown samples, were analyzed for pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter*, as well as generic *E. coli* (an indicator of fecal contamination). *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter* were not detected in any of the samples tested (Table 6). Generic *E. coli* counts were not found to exceed 100 CFU/g in any of the samples. Therefore, these samples were all (100%) assessed as satisfactory (Table 6).

Table 6 Summary of Results of Whole Leafy Vegetable Samples

(Samples were analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, and generic *E. coli*.)

Product Origin	Production Practice	Number of Samples	Assessment		
			Investigative	Unsatisfactory	Satisfactory
Domestic	Conventional	238	0	0	238
	Organic	82	0	0	82
Total		**320**	**0**	**0**	**320 (100%)**

3.2 Fresh-cut Leafy Vegetable Samples Analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes* and generic *E. coli*

In the fresh-cut leafy vegetable group, samples were analyzed for pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, and *L. monocytogenes*, as well as generic *E. coli* (Table 7). A total of 650 fresh-cut samples (99.5%) were assessed as satisfactory, one sample (0.2%) was assessed as investigative due to an elevated level of generic *E. coli*, and two samples were assessed as unsatisfactory due to the presence of *L. monocytogenes*.

Table 7 Summary of Results of Fresh-cut Leafy Vegetable Samples

(Samples were analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, and generic *E. coli*.)

Product Origin	Production Practice	Number of Samples	Assessment		
			Investigative	Unsatisfactory	Satisfactory
Imported	Conventional	57	0	0	57
	Organic	335	0	0	335
Domestic	Conventional	219	1	1	217
	Organic	42	0	1	41
Total		**653**	**1 (0.2%)**	**2 (0.3%)**	**650 (99.5%)**

E. coli O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter* were not detected in any of the samples tested. An elevated level of generic *E. coli* (240 CFU/g) was found in one sample (1/653, 0.2%) and this sample was assessed as investigative, as the *E. coli* counts were elevated (100 -1000 CFU/g) but below the unsatisfactory threshold. Further evaluation of the sample resulted in no immediate follow-up sampling.

L. monocytogenes was detected in two samples (2/653, 0.3%) and the levels were all below 5 CFU/g (Table 8). Further evaluation resulted in unsatisfactory assessment as their stated shelf-life was ≥ 5 days. However, there were no product recalls since these products were passed their stated shelf-life when enumeration results were available. The CFIA conducted follow-up activities for these contaminated samples including a joined inspection with the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) at one of the processing facility. No reported illnesses were found to be associated with the contaminated products during this survey.

Table 8 Summary of Unsatisfactory and Investigative Samples

Product Origin	Product Type/Production Practice	Reason for Assessment
Domestic	Shredded cabbages/Conventional	Unsatisfactory: *L. monocytogenes* detected, enumeration <5 CFU/g, stated shelf-life of the product >5 days
	Salad mix/Organic	Unsatisfactory: *L. monocytogenes* detected, enumeration <5 CFU/g, stated shelf-life of the product >5 days
	Lettuce romaine (fresh-cut)/Conventional	Investigative: generic *E. coli* counts = 240 CFU/g

3.3 Head Lettuce Samples Analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and generic *E. coli*

A combination of three pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella, Shigella,* and the indicator of fecal contamination generic *E. coli,* were tested in the head lettuce sample group (Table 9). *E. coli* O157:H7/NM, *Salmonella* and *Shigella* were not detected in any of the samples tested. Generic *E. coli* counts were not found to exceed 100 CFU/g in all samples. All head lettuce samples (100%) were assessed as satisfactory.

Table 9 Summary of Results of Head Lettuce Samples

(Samples were analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella,* and generic *E. coli.*)

Product Origin	Production Practice	Number of Samples	Assessment		
			Investigative	Unsatisfactory	Satisfactory
Imported	Conventional	266	0	0	266
	Organic	4	0	0	4
Domestic	Conventional	294	0	0	294
Total		**564**	**0**	**0**	**564 (100%)**

3.4 Result Summary by Targeted Microorganism

The results of all testing are summarized according to targeted microorganisms (Table 10).

Table 10 Result Summary by Targeted Microorganism

Targeted Microorganism	**Number of Unsatisfactory Samples/ Number of Samples Tested** (Investigative results are indicated in brackets)		
	Imported Samples	**Domestic Samples**	**Total**
Generic *E. coli*	0/662	0(1)*/875	0(1)*/1537
***E. coli* O157**	0/662	0/875	0/1537
Salmonella	0/662	0/875	0/1537
Shigella	0/662	0/875	0/1537
Campylobacter	0/392	0/581	0/973
L. monocytogenes	0/392	2**/261	2**/653

* One domestic sample was found to have an elevated level of generic *E. coli* and the sample was assessed as investigative.

** Two domestic fresh-cut samples were found to be contaminated with low levels (< 5 CFU/g) of *L. monocytogenes* and these samples were assessed as unsatisfactory due to their stated shelf-life >5 days.

4 Discussion and Conclusion

The results of the 2011/12 survey indicate that enteric bacterial pathogens *E. coli* O157, *Salmonella, Shigella* and *Campylobacter* were not detected in any of the leafy vegetable samples analyzed. However, the environmental bacterial pathogen *L. monocytogenes* was found in two of the fresh-cut leafy vegetable samples. In addition, one sample was found to have an elevated, yet marginally acceptable, level of generic *E. coli* (>100 but ≤1,000 CFU/g).

The two samples that were positive for *L. monocyotogenes* (at levels ≤ 5 CFU/g) were assessed as unsatisfactory as the products stated shelf-life was longer than five days. The CFIA conducted follow-up activities for these contaminated products including a joint inspection with OMAFRA at one of the processing facility. No products were recalled as they were passed their shelf-life by the time the results were confirmed. There were no reported illnesses associated with the *L. monocytogenes* contaminated products during this survey.

In this survey, the presence of *L. monocytogenes* in fresh-cut leafy vegetable samples occurred at a very low rate (0.3%) and at very low levels (<5 CFU/g). Similar prevalence of *L. monocytogenes* in retail fresh-cut leafy vegetable samples were reported at 0.7%, 0.9%, and 0.9% from studies conducted in the U.S. (2966 bagged salad samples) (22),

Spain (161 fresh-cut lettuce and salad samples) (23) and Brazil (133 minimal processed leafy vegetable samples) (24), respectively.

The overall findings of this survey suggest that the vast majority of fresh leafy vegetables in the Canadian market are produced and handled under acceptable GAPs and GMPs. However, contamination of fresh-cut RTE leafy vegetables with *L. monocytogenes* can occur sporadically, which may represent a food safety risk for high-risk population groups (e.g., pregnant women, older adults, people with weakened immune systems).

The food industry and retail sectors in Canada are ultimately responsible for the food they produce and sell, and individual consumers are responsible for the safe handling of the food they have in their possession. However, the CFIA regulates the food industry, provides oversights and promotes safe handling of foods throughout the food production chain. The CFIA will continue its surveillance activities and inform stakeholders of its findings.

5 Acknowledgment

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Appendix A: List of Acronyms

CDC: Centre for Disease Control and Prevention

CFIA: Canadian Food Inspection Agency

CFU: colony forming unit

E. coli: *Escherichia coli*

FAO: Food and Agriculture Organization of the United Nations

FDA: *Food and Drugs Act*

FDR: *Food and Drug Regulations*

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

HC: Health Canada

MPN: Most Probable Number

PCR: Polymerase Chain Reaction

PHAC: Public Health Agency of Canada

spp.: species

USFDA: United States Food and Drug Administration

WHO: World Health Organization

°C: Degree Celsius

g: gram

Appendix B: Global Foodborne Disease Outbreaks Associated With Leafy Green Vegetables Contaminated with Bacterial Pathogens (1998 - 2011)*

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Number of Deaths)
1	1998	April	1999 Int. J. Food. Microbiol 49:103-6	Japan	N/A	*Clostridium perfringens*	Spinach	30	
2	1998	June	CDC	USA	Minnesota	*Campylobacter jejuni*	Lettuce	300	
3	1998	October	Ann. Rheum. Dis. 62(9):866-869, 2003	Finland	Multiple	*Yersinia pseudotuberculosis*	Lettuce, iceberg	38	13
4	1999	February	CDC	USA	Nebraska	*Escherichia coli* O157:H7	Lettuce, iceberg	72	
5	1999	February	CDC	USA	Nebraska	*Escherichia coli* O157:H9	Lettuce, iceberg	65	
6	1999	September	Epi. & Infect. 132:43-49, 2003	Sweden	N/A	*Escherichia coli* O157	Lettuce	13	2
7	1999	September	CDC	USA	Multiple	*Escherichia coli* O157	Lettuce, romaine	14	
8	1999	October	CDC	USA	Pennsylvania	*Escherichia coli* O153:H50	Lettuce, romaine	40	
9	1999	October	CDC	USA	Multiple	*Escherichia coli* O157:H7	Lettuce, romaine	46	7
10	2000		NML, Annual Summary	Canada	Nova Scotia	*Escherichia coli* O157:H7	Spinach	11	
11	2000		CDR Enteric Archives 2001	England	N/A	*Campylobacter*	Lettuce	18	
12	2000		Clin. Micro. & Infect. 9(8) 839-845, 2003	Multiple	N/A	*Salmonella* Typhimurium DT204b	Lettuce, iceberg	392	61
13	2000	May	CDC	USA	Connecticut	*Campylobacter jejuni*	Lettuce	13	
14	2000	August	Epi. & Infect. 130;169-178, 2003	UK	N/A	*Salmonella* Typhimurium DT104	Lettuce	361	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Number of Deaths)
15	2001	May	Infect. Dis. News Brief, 7 Sept 2001	Australia	Queensland	*Salmonella* Bovismorbificans	Lettuce, iceberg	41	
16	2001	May	Infect. Dis. News Brief, 9 Jul 2001	Canada	Multiple	Shigella sonnei	Spinach	31	1
17	2001	November	Food Safety Network Sept. 18 2006	USA	Texas	*Escherichia coli* O157:H7	Lettuce	20	
18	2001	December	CDC	USA	Virginia	*Clostridium perfringens*	Spinach	33	
19	2002	July	FDA	USA	Washington	*Escherichia coli* O157:H8	Lettuce, romaine	29	
20	2002	November	CDC	USA	Illinois	*Escherichia coli* O157:H7	Lettuce	13	
21	2002	December	Food Safety Network Sept. 18 2006	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	3	
22	2003	September	CDC	USA	California	*Escherichia coli* O157:H7	Lettuce	51	
23	2003	October	CDC	USA	California	*Escherichia coli* O157:H7	Spinach	46	7 (1)
24	2003	November	CDC	USA	California	*Salmonella* Enteritidis	Lettuce	14	
25	2004	July	CDC	USA	Multiple	*Salmonella* Newport	Lettuce	97	
26	2004	August	New Hampshire Dept. of Health & Human Services	USA	New Hampshire	Salmonella	Lettuce	9	
27	2004	September	Epi. & Infect. 137(10):1449-1456, 2009	England	N/A	*Salmonella* Newport	Lettuce	677	
28	2004	November	J. Foodborne Pathogens & Dis. 5(2):165-173	Norway	N/A	*Salmonella* Thompson	Lettuce	21	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Number of Deaths)
29	2004	November	Food Safety Network Sept. 18 2006	USA	New Jersey	*Escherichia coli* O157:H7	Lettuce	6	
30	2005		European Food Safety Authority	UK	N/A	*Salmonella* Typhimurium	Lettuce, iceberg	71	
31	2005	April	CDC	USA	Oregon	*Salmonella* Paratyphi B var Java	Lettuce	10	
32	2005	May	Eurosurveillance Weekly 10 (44), 2005	Finland	N/A	*Salmonella* Typhimurium DT104	Lettuce	60	
33	2005	August	CDR Weekly Vol. 15 No. 36	England	N/A	*Salmonella* Typhimurium DT104	Lettuce	71	
34	2005	August	Eurosurveillance Weekly 10(9), 2005	Sweden	N/A	*Escherichia coli* O157	Lettuce	135	
35	2005	September	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	34	13
36	2005	September	Bites (Kansas State)	USA	Multiple	*Escherichia coli* O157:H7	Spinach	204	
37	2006	January	CDC	USA	Oregon	*Shigella sonnei*	Lettuce	35	7
38	2006		European Food Safety Authority	UK	N/A	*Salmonella* ajioba	Lettuce	153	11
39	2006	June	Weber-Morgan Health Dept.	USA	Utah	*Eschericha coli* O121:H19	Lettuce	73	
40	2006	August	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	3	
41	2006	September	CFIA	Canada	Ontario	*Escherichia coli* O157:H7	Lettuce	30	5
42	2006	October	FSNet Jan 9, 2007	USA	North Carolina	*Escherichia coli*	Lettuce	9	3
43	2006	November	CDC	USA	Tennessee	*Salmonella* Javiana	Lettuce, iceberg	16	7

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Number of Deaths)
44	2006	November	CDC	USA	New York	*Escherichia coli* O157:H7	Lettuce	20	14
45	2006	November	Minnesota Dept. of Health	USA	Minnesota	*Escherichia coli* O157:H7	Lettuce	32	
46	2006	December	CFIA	Canada	Ontario	*Salmonella* Oranienburg	Spinach	3	
47	2006	December	New Jersey Dept. of Health and Senior Services	USA	New Jersey	*Escherichia coli* O157	Lettuce	37	
48	2007	February	CDC	USA	Multiple	*Salmonella* Typhimurium	Lettuce	76	4
49	2007	March	CDC	USA	Hawaii	*Escherichia coli* O157:H7	Lettuce	8	5
50	2007	June	CDC	USA	Alabama	*Escherichia coli* O157:H7	Lettuce	26	11 (1)
51	2007	July	Thu 20 Dec 2007 Eurosurveillance Weekly	Sweden	N/A	*Salmonella* Java	Spinach	172	46
52	2007	July	CDC	USA	California	*Shigella sonnei*	Lettuce	72	9
53	2007	September	Eurosurveillance weekly 12(11) 2007	Iceland	N/A	*Escherichia coli* O157	Lettuce, iceberg	9	7
54	2007	September	Eurosurveillance 11 Dec. 2008	Netherlands		*Escherichia coli* O157	Lettuce	50	
55	2008	June	Washington Dept. of Health	USA	Washington	*Escherichia coli*	Lettuce	10	2
56	2008	August	Michigan Dept. of Community Health	USA	Michigan	*Escherichia coli* O157:H7	Lettuce, iceberg	36	8
57**	2008	October	References (10, 11)	Canada	Ontario	*Escherichia coli* O157:H7	Lettuce, iceberg	3	
58	2008	October	Wellington-Dufferin-Guelph Public Health	Canada	Ontario	*Escherichia coli* O157:H7	Lettuce, romaine	148	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Number of Deaths)
59	2009	July	Public Health Division in Oregon	USA	Multiple	*Salmonella*	Lettuce	124	2
60	2010	March	CDC	USA	Multiple	*Escherichia coli* O145	Lettuce, romaine	33	12
61	2011	March	Eurosurveillance, 16:19, 2011	Norway		*Yersinia enterocolitica* O:9	Lettuce	21	
62	2011	October-December	CDC	USA	Missouri	*E.coli* O157:H7	Lettuce, romaine	58	33

* Information in this appendix was prepared by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC (Public Health Agency of Canada)

**References (10, 11).

Appendix C: Summary of Global Foodborne Disease Outbreaks Associated With Leafy Green Vegetables Contaminated with Bacterial Pathogens (1998-2011)

Bacterial Pathogens	Number of Outbreaks	Percentage of Outbreaks
E. coli O157	28	45.2
Other *E. coli*	5	8.1
Salmonella	19	30.6
Shigella	3	4.8
Campylobacter	3	4.8
Clostridium perfringens	2	3.2
Yersinia	2	3.2
Total	**62**	**100.0**

Summarized according to appendix B

Appendix D: Analytical Methods Used for Microbial Analysis

Microbial Analysis	Method Identification Number (Date Issued)	Title of Method*
***E. coli* O157:H7/NM**	MFLP-30 (May 2003, Supplement 1 May 2005 & Supplement 2 November 2006)	The Dupont Qualicon Bax® System Method for the Detection of *E. coli* O157:H7 in Raw Beef and Fruit Juice
	MFLP-80 (March 2008)	Isolation of *E. coli* O157:H7 or NM in Foods
***Campylobacter* spp.**	MFLP-46 (Modified**)	Isolation of Thermophilic *Campylobacter* from Foods
L. monocytogenes	MFLP 28	The Qualicon Bax® System Method for the Detection of *Listeria monocytogenes* in a Variety of Food
	MFHPB-30 (April 2002)	Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples
	MFLP-74 (January 2001, Supplement March 2002)	Enumeration of *Listeria monocytogenes* in Food
	Appendix L (August 2005)	Confirmation Steps for Methods for The Detection of *Listeria* spp. In Foods And Environmental Samples
***Salmonella* spp.**	MFLP-29 *** (July 2007, modified)	The Qualicon Bax® System Method for the Detection of *Salmonella* in a Variety of Food and Environmental Samples
	MFHPB-20 (March 2009)	Methods for the Isolation and Identification of *Salmonella* from Foods and Environmental Samples
***Shigella* spp.**	MFLP-26 (February 2006)	Detection of *Shigella* spp. In Foods by the Polymerase Chain Reaction (PCR)
	MFLP-25 (March 2006)	Isolation and Identification of *Shigell*a spp. From Foods
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in Foods

	MFHPB-27 (September 1997)	Enumeration of *Escherichia coli* in Foods by the Direct Plating (DP) Method

**Compendium of Analytical Methods (26).*

** MFLP-46 was performed with the following modification to include wash with peptone water to collect *Campylobacter* from the samples, followed by enrichment.

*** MFLP-29 was performed as written with the following modification: Secondary enrichment was performed as outlined for cantaloupes, i.e., transferred from buffered peptone broth as specified to RVS and TBG broths (Rappaport-Vassiliadis Soya Peptone broth and Tetrathionate Brilliant Green broth) and incubated for 24 ± 2 h at 42.5°C. After incubation 2 ml from each of RVS and TBG are combined to one sample and proceed with step 7.3.1.4 of the method.