Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Targeted Survey

REPORT

2012/13 - 2013/14 Targeted Surveys

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

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Canada

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Executive Summary

Targeted surveys are used by the Canadian Food Inspection Agency (CFIA) to focus its surveillance activities on areas of highest risk. The information gained from these surveys provides both support for the prioritization of the Agency's activities to areas of greater concern and scientific evidence to address areas of lesser concern. Originally started under the Food Safety Action Plan (FSAP), targeted surveys have been incorporated into the CFIA's regular surveillance activities as a valuable tool for generating essential information on certain hazards in foods, identifying/characterizing new and emerging hazards, informing trend analysis, prompting/refining human health risk assessments, highlighting potential contamination issues as well as assessing and promoting compliance with Canadian regulations.

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.

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. Over the course of a baseline study between the 2008/09 and 2013/14 fiscal years, approximately 12,000 leafy vegetable samples were collected from Canadian retail locations and tested for various pathogens of concern.

The main objectives of the 2012/13 and 2013/14 surveys were to generate baseline surveillance data on bacterial pathogens *Escherichia coli* (*E. coli*) O157:H7/NM (non-motile), *Salmonella*, *Shigella*, *Campylobacter*, *Listeria monocytogenes* (fresh-cut only) and Verotoxin-producing *E. coli* (VTEC) (fresh-cut only), 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 2,977 fresh leafy vegetable samples, including whole and fresh-cut samples, were collected and tested for the targeted bacteria. Most samples (99.5%) were assessed as satisfactory. One of the fresh-cut samples (0.1%) was found to be contaminated with *L. monocytogenes*. The CFIA conducted appropriate follow-up activities for the contaminated product, including a product recall. No reported illnesses were found to be associated with the contaminated products identified during this survey. In addition, four samples had high levels (>1,000 Most Probable Number (MPN)/g) of generic *E. coli* and six samples had elevated, yet marginally acceptable levels (100 – 1,000 MPN/g) of generic *E. coli*. Further evaluations of these samples resulted in no immediate

follow-up activities. Generic *E. coli* is an indicator used by the CFIA to assess general sanitation and hygiene practices throughout the production chain. 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 Targeted Surveys

The Canadian Food Inspection Agency (CFIA) monitors both domestic and imported foods for the presence of allergenic, microbiological, chemical, and physical hazards. One of the tools used to maintain this oversight are targeted surveys, which are a means to establish baseline information on specific hazards and to investigate emerging risks. Targeted surveys are part of the Agency's core activities along with other surveillance strategies, which include the National Chemical Residue Monitoring Program (NCRMP), the National Microbiological Monitoring Program (NMMP), and the Children's Food Project (CFP). The surveys are complementary to other CFIA surveillance activities in that they examine hazards and/or foods that may not be routinely included in these monitoring programs.

Targeted surveys are used to gather information regarding the possible occurrence or prevalence of hazards in defined food commodities. These surveys generate essential information on certain hazards in foods, identify or characterize new and emerging hazards, inform trend analysis, prompt or refine human health risk assessments, assess compliance with Canadian regulations, highlight potential contamination issues, and/or influence the development of risk management strategies as appropriate.

Due to the vast number of hazard and food commodity combinations, it is not possible, nor should it be necessary, to use targeted surveys to identify and quantify all hazards in foods. To identify food-hazard combinations of greatest potential health risk, the CFIA uses a combination of scientific literature, the media, and/or a risk-based model developed by the Food Safety Science Committee, a group of federal, provincial and territorial subject matter experts in the area of food safety.

These targeted surveys (2012/13 and 2013/14) represents part of the collection of approximately 12, 000 leafy green vegetable samples over five years (2009/10 to 2013/14) 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.

1.2 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) [1] and the *Recommended International Code of Practice -*

General Principles of Food Hygiene (CAC/RCP 1-1969) [2]. 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) [3] and the *Food and Drug Regulations* (FDR) [4], 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* [5] under the *Canada Agricultural Products Act* [6]. 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 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 2014, 81 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 [7, 8] (information based on data compiled by the Public Health Agency of Canada (PHAC), Appendix B & C). The frequency of outbreaks associated with *Escherichia coli* (*E. coli*) O157:H7 and *Salmonella* were higher than the frequency due to other pathogenic bacteria (Appendix B & C). It is noted that the outbreaks associated with *E. coli* O157:H7 and other serogroups of pathogenic *E. coli* accounted for 59.2% of all the reported pathogenic bacteria associated outbreaks (Appendix 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.

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 [9]. 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 [10, 11].

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 [12]. 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 [13], fresh leafy vegetables have been selected for targeted surveillance. The overall objective of this multi-year study was to gather baseline information on the occurrence of various pathogens (bacteria, viruses and parasites) of concern in leafy vegetables available to Canadians at retail. The 2012/13 and 2013/14 surveys in this report were part of the information collection of the multi-year surveys (2009/10-2013/14) on leafy vegetables and had 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, conventionally and/or organically produced leafy vegetables.

2.2 Targeted Microorganisms

2.2.1 Bacterial Pathogens of Concern

Bacterial pathogens *Salmonella* and *E. coli* O157:H7 are found naturally in the intestines of animals, such as poultry and cattle, respectively [14]. 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 illnesses related to these bacteria [7]. 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 [15].

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 [14].

Similarly to *Salmonella*, 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. [16] and Canada [17]. Raw poultry and unpasteurized (raw) milk are major sources of contaminated food. However, vegetables were also found, sporadically, to be contaminated with *Campylobacter* [14].

Verotoxin-producing *E. coli* (VTEC), such as O157 and other non-O157 *E. coli* serogroups (e.g., O26, O103, O111, O117, O121 and O145) produce verocytotoxins that can cause human illness [18]. Outbreaks of foodborne illnesses associated with VTEC are often linked to consumption of contaminated beef. Other than beef, contaminated vegetables have also been found to be responsible for numerous VTEC associated foodborne outbreaks (e.g., lettuce, spinach, and sprouts) [14].

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 a wide range of growth temperatures (i.e., -0.4 to 45ºC) that includes the typical refrigeration temperature of 4°C [19]. 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 [19].

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., 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. Iceberg lettuce was excluded from the 2012/13 survey.

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 2012/13 and 2013/14 fiscal years (April 1, 2012 to March 31, 2014). Domestic samples were mainly collected during the summer months and imported samples were collected primarily in the fall, winter, and spring months. Samples that were labeled as organic at retail were identified as "organic". 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 [20, 21, 22] and by other federal partners such as the Public Health Agency of Canada (PHAC) under the FoodNet retail surveillance [23].

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 Analytical Methods and Assessment Guidelines

Samples were analysed using the analytical methods as published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods [24] (Appendix D), except for the VTEC method. These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of fresh fruits and vegetables, including leafy herbs.

The assessment criteria presented below (Table 1 and Table 2) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* [25] and associated methods published in Health Canada's *Compendium of Analytical Methods* [24], as well as Health Canada's "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (2011)" [19].

Table 1 Assessment Guidelines for Bacterial Pathogens in Leafy Vegetables

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
***E. coli* O157:H7/NM** (MFLP-30 and MFLP-80 if required for confirmation)	Absent in 25 g	Present in 25 g
Salmonella* spp.* (MFLP-29 modified and MFHPB-20 if required for confirmation)	Absent in 25 g	Present in 25 g
Shigella* spp. * (MFLP-26 and MFLP-25 if required for confirmation)	Absent in 25 g	Present in 25 g
Campylobacter* spp.* (MFLP-46 modified)	Absent in 25 g	Present in 25 g
VTEC (CFIA and HC published method[26, 27, 28])	Absent in 25 g	Present in 25 g

* *Compendium of Analytical Methods* [24].

**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 2 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 if required for enumeration)	Absent in 25 g	Detected and ≤ 100 CFU/g	> 100 CFU/g

* Compendium of Analytical Methods [24]

** 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 product in question.

Samples assessed as investigative for *L. monocytogenes* (≤ 100 Colony Forming Units (CFU)/g) in these surveys 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 [19].

2.5 Limitations

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 Sample Distribution

3.1.1 Sample Distribution by Targeted Microorganism Group

As per survey design, three groups of leafy vegetable samples were collected and analyzed for various combinations of targeted microorganisms (Table 3). Due to the limitation of the laboratory capacity, only Group III fresh-cut leafy vegetable samples were tested for VTEC.

Table 3 Sample Distribution by Targeted Microorganism Group

Objective Group	Targeted Microorganism	Products Origin	Production Practice	Number of Samples
Group I (whole leafy vegetables)	*E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, and generic *E. coli*	Imported	Conventional	605
			Organic	557
		Domestic	Conventional	495
			Organic	104
		Subtotal		1761
Group II (fresh-cut leafy vegetables)	*E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, and generic *E. coli*	Imported	Conventional	698
			Organic	182
		Domestic	Conventional	118
			Organic	0
		Subtotal		998
Group III (fresh-cut leafy vegetables)	VTEC, *E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, and generic *E. coli*	Imported	Conventional	190
			Organic	0
		Domestic	Conventional	28
			Organic	0
		Subtotal		218

3.1.2 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 4).

Table 4 Sample Distribution by Country of Origin

Country of Origin	Group I (whole)	Group II (fresh-cut)	Group III (fresh-cut)	Grand Total
	Number of Samples (%)	Number of Samples (%)	Number of Samples (%)	Number of Samples (%)
Canada	599 (34%)	118 (12%)	28 (13%)	745 (25%)
Guatemala	0	1	1	2
Mexico	50	2	2	54
United States	1111 (63%)	863 (87%)	187 (86%)	2161 (73%)
Un-identified	1	14	0	15
Imported-subtotal	*1162 (66%)*	*88 (88%)*	*190 (87%)*	*2232 (75%)*
Total	**1761 (100%)**	**998 (100%)**	**218 (100%)**	**2977 (100%)**

3.1.3 Sample Distribution by Product Type

The product types were tabulated for each defined leafy vegetable group (Table 5). Except for iceberg lettuce, which was excluded from the scope of the leafy vegetable survey in 2012/13, other types of leafy vegetables were collected from the market based on product availability.

Table 5 Product Type in Each Group of Leafy Vegetable Samples

Product Type	Group I (whole)	Group II (fresh-cut)	Group III (fresh-cut)
	Number of Samples (%)	Number of Samples (%)	Number of Samples (%)
Arugula	129	0	0
Chard	9	2	0
Chicory	0	0	0
Collard	7	3	0
Dandelion	1	0	0
Endives	13	0	0
Kale	30	3	0
Spinach	627	0	0
Spring mix	181	0	0
Spring mix with herbs	7	0	0
Watercress	11	0	0
Other*	1	0	0
Subtotal	*1016 (57.7%)*	*8 (0.8%)*	*0*
-Boston/butter lettuce	115	0	0
-Iceberg lettuce	4	3	0
-Green leafy lettuce	18	0	0
-Mache	15	0	0
-Red leafy lettuce	12	0	0
-Romaine	376	131	17
-Mixed lettuce	30	24	0
-Other unspecified	11	6	39
Subtotal Lettuce	*581 (33.0%)*	*164 (16.4%)*	*56 (25.7%)*
-Baby romaine**	36	0	0
-Baby lettuce blend	45	0	0
-Baby spinach blend	17	0	0
-Iceberg blend	0	66	16
-Iceberg & Romaine blend	0	188	40
-Romaine blend	22	345	48
-Other/unspecified	32	55	12
Subtotal Salad Mix	*152 (8.6%)*	*654 (65.5%)*	*116 (53.2%)*
-Baby lettuce blend	1	0	0
-Baby Spinach blend	8	0	0
-Iceberg & Romaine blend	0	39	10
-Romaine blend	0	123	30
-Other/unspecified	3	10	6
Subtotal Salad Kit	*12 (0.7%)*	*172 (17.2%)*	*46 (21.1%)*
Total	**1761 (100%)**	**998 (100%)**	**218 (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.

** Baby leafy vegetables are classified as whole leafy vegetables, but not fresh-cut leafy vegetables by the CFIA.

3.2 Assessment Results

3.2.1 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 1,761 samples including imported domestically produced, 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). Bacterial pathogens were not found in any of the samples tested (Table 6). Generic *E. coli* counts were found to exceed 1,000 MPN/g in four samples (0.2%) and to be between 100 and 1,000 MPN/g in five samples (0.3%). Other samples (99.5%) were 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
			Number of Samples (%)	Number of Samples (%)	Number of Samples (%)
Imported	Conventional	605	0	0	605
	Organic	557	1	0	556
Domestic	Conventional	495	3	2	490
	Organic	104	1	2	101
Total		**1761**	**5 (0.3%)**	**4 (0.2%)**	**1752 (99.5%)**

The samples containing high levels (>1,000 MPN/g) and elevated levels (100 – 1,000 MPN/g) of generic *E. coli* were assessed as unsatisfactory and investigative, respectively (Table 7). Further evaluations of the unsatisfactory and investigative samples resulted in no immediate follow-up activities. These samples were either whole leafy vegetables or packaged baby leafy vegetables labelled with instructions of washing before consumption.

Table 7 Summary of Unsatisfactory and Investigative Whole Leafy Samples

Product Origin	Product /Production Practice	Reason for Assessment
Domestic	Spinach/ Conventional	Unsatisfactory: generic *E. coli* >1600 MPN/g
	Spring mix/Conventional	Unsatisfactory: generic *E. coli* counts >1600 MPN/g
	Kale/Organic	Unsatisfactory: generic *E. coli* counts >1600 MPN/g
	Spring mix/Organic	Unsatisfactory: generic *E. coli* counts >1600 MPN/g
	Chard/Conventional	Investigative: generic *E. coli* counts = 540 MPN/g
	Arugula/Organic	Investigative: generic *E. coli* counts = 540 MPN/g
	Spinach/Conventional	Investigative: generic *E. coli* counts = 350 MPN/g
	Salad mix/Conventional	Investigative: generic *E. coli* counts = 170 MPN/g
Imported	Kale/Organic	Investigative: generic *E. coli* counts = 240 MPN/g

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

In this 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 8). *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter* were not detected in any of the samples tested. One sample (0.1%) was assessed as investigative due to an elevated level (100 - 1,000 MPN/g) of generic *E. coli*, and one sample was assessed as unsatisfactory due to the presence of *L. monocytogenes.* The rest of the samples (99.8%) were assessed as satisfactory.

Table 8 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
			Number of Samples (%)	Number of Samples (%)	Number of Samples (%)
Imported	Conventional	698	0	0	698
	Organic	182	1	1	180
Domestic	Conventional	118	0	0	118
	Organic	0	0	0	0
Total		**998**	**1 (0.1%)**	**1 (0.1%)**	**996 (99.8%)**

Further evaluation of the sample with an elevated level of generic *E. coli* (540 MPN/g) resulted in no immediate follow-up sampling (Table 9).

L. monocytogenes was detected in one sample (0.1%) at a level of 80 CFU/g (Table 9). Further evaluation resulted in an unsatisfactory assessment [19]. The CFIA conducted follow-up activities including a product recall. No reported illnesses were found to be associated with the contaminated products identified during this survey.

Table 9 Summary of Unsatisfactory and Investigative Fresh-cut Leafy Vegetable Samples

Product Origin	Product/Production Practice	Reason for Assessment
Imported	Italian blend/Organic	Unsatisfactory: *L. monocytogenes* was detected at a level of 80 CFU/g. The sample also had an elevated level of generic *E. coli* (240 MPN/g)
	Salad mix/Organic	Investigative: generic *E. coli* counts = 540 MPN/g

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

Pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella, Shigella, Campylobacter, L. monocytogenes,* VTEC, and the indicator of fecal contamination generic *E. coli*, were tested in this fresh-cut sample group (Table 10). Pathogens were not detected in any of the samples tested. Generic *E. coli* counts were not found to exceed 100 MPN/g in any samples. All fresh-cut samples (100%) were assessed as satisfactory.

Table 10 Summary of Results of Fresh-cut Leafy Vegetable Samples

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

Product Origin	Production Practice	Number of Samples	Assessment		
			Investigative	Unsatisfactory	Satisfactory
Imported	Conventional	190	0	0	190
Domestic	Conventional	28	0	0	28
Total		**218**	**0**	**0**	**218 (100%)**

3.3 Summary of Results by Targeted Microorganisms

The results of all testing are summarized by targeted microorganisms in Table 11.

Table 11 Result Summary by Targeted Microorganisms

Targeted Microorganism	Number of Unsatisfactory Samples/ Number of Samples Tested (Number of samples with investigative assessment are indicated in bracket*)		
	Imported Samples	Domestic Samples	Total
Generic *E. coli*	0(2*)/2232	4(4*)/745	4(6*)/2977
***E. coli* O157**	0/2232	0/745	0/2977
Salmonella	0/2232	0/745	0/2977
Shigella	0/2232	0/745	0/2977
Campylobacter	0/2232	0/745	0/2977
L. monocytogenes	1/1070	0/146	1/1216
VTEC	0/190	0/28	0/218

* Elevated levels of generic *E. coli* were found in these samples.

4 Discussion and Conclusion

The results of the 2012/13 and 2013/14 surveys indicate that enteric bacterial pathogens *E. coli* O157:H7, *Salmonella*, *Shigella*, *Campylobacter*, and VTEC were not detected in any of the leafy vegetable samples analyzed (2,977 samples). The environmental bacterial pathogen *L. monocytogenes* was found in one of the fresh-cut leafy vegetable samples. In addition, high levels (>1,000 MPN/g) of generic *E. coli* were found in four whole leafy vegetable samples; and elevated, yet marginally acceptable levels (100 - 1,000 MPN/g) of generic *E. coli* were found in six samples.

The *L. monocyotogenes* contaminated sample was further evaluated and assessed as unsatisfactory [19]. The CFIA conducted follow-up activities including a product recall. 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 (0.1%) and at a level less than 100 CFU/g. Comparable prevalence of *L. monocytogenes* in retail fresh-cut leafy vegetable samples were reported from studies conducted in the U.S. (0.7% in 2,966 bagged salad samples) [20], Spain (0.9% in 161 fresh-cut lettuce and salad samples) [21] and Brazil (0.9% in 133 minimal processed leafy vegetable samples) [22].

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).

While 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, the CFIA regulates the food industry, provides oversight 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*

FSAP: Food Safety Action Plan

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

HC: Health Canada

L. monocytogenes: *Listeria monocytogenes*

MPN: Most Probable Number

PCR: Polymerase Chain Reaction

PHAC: Public Health Agency of Canada

spp.: species

USFDA: United States Food and Drug Administration

VTEC: Verotoxin-producing *E. coli*

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 - 2014)*

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (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 (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	
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	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Deaths)
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
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	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Deaths)
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	
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	May	CDC line list 2011	USA	New York	*E.coli* O6:H16	Spinach	19	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Deaths)
63	2011	October-December	CDC	USA	Missouri	*E.coli* O157:H7	Lettuce, romaine	58	33
64	2012	March	CDC line list 2012	USA	Pennsylvania	*B. cereus*	Lettuce leaf	14	
66	2012	May	CDC line list 2012	USA	Georgia	*E.coli* O157:H7	Lettuce, iceberg	2	2(1)
66	2012	May	Bites May 1/13	Canada	Alberta	*E.coli* O157:H7	Lettuce	3	(1)
67	2012	June	CDC line list 2012	USA	Multiple states	*E.coli* O157:H7	Lettuce, romaine	52	
68	2012	July	CDC line list 2012	USA	Multiple state	*S.* Newport	Lettuce romaine	15	
69	2012	September	CDC line list 2012	USA	Pennsylvania	*E.coli* O157:H7	Lettuce, romaine	9	7
70	2012	November	CDC	USA		*E.coli* O157:H7	Spinach &spring mix	33	13(0)
71	2012		Public Health in Hamilton county	USA	Ohio	*E.coli*	Lettuce	5	0
72	2012	November	CDC line list 2012	USA	Massachusetts	*E.coli* O157:H7	lettuce	8	4
73	2012	November	CDC line list 2012	USA	Washington	*E.coli* O157:H7	Spinach	4	1
74	2012		CDC line list 2012	USA	Multiple states	*E.coli* O145	lettuce	16	6
75	2012	December	CCDR 2012, 40-S1	Canada	Multiple provinces in Canada	*E.coli* O157:H7	Lettuce	31	13 (0)
76	2012		NY state Health Department	USA	New York	*E.coli* O157:H7	Spinach	16	4
77	2013		Norway annual Report	Norway	Norway	*S.* Coeln	Lettuce	26	0
78	2013	September	UK annual report 2013	United Kingdom	UK	*E.coli* O157:H7	Watercress	6	
79	2014	June	HPR 8(27), 2014	United Kingdom	UK	*E. coli* O157	Lettuce	50	0

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Deaths)
80	2014		Health Protection Report 8(31)2014	United Kingdom	UK	*E. coli* O96	lettuce	15	0
81	2014	September	ProMED Digest 28(21&48)2014	New Zealand	New Zealand	*Y.pseudotuberculosis*	lettuce	127	

* 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 (1998-2014)

Bacterial Pathogens	Number of Outbreaks	Percentage of Outbreaks
E. coli O157	39	48.1
Other *E. coli*	9	11.1
Salmonella	21	25.9
Shigella	3	3.7
Campylobacter	3	3.7
Clostridium perfringens	2	2.5
Yersinia	3	3.7
B. cereus	1	1.2
Total	**81**	**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 (November, 2012)	Detection of *Escherichia coli* O157:H7 in select foods using the BAX® System *E. coli* O157:H7 MP
	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 (November 2011)	The Qualicon Bax® System Method for the Detection of *Listeria monocytogenes* in a Variety of Food
	MFHPB-30 (February 2011)	Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples
	MFLP-74 (February 2011)	Enumeration of *Listeria monocytogenes* in Food
***Salmonella* spp.**	MFLP-29 *** (June 2012, 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
VTEC	CFIA and HC Published Methods ****	Detection of Verotoxin-Producing *Escherichia coli* in Food
		A Cloth-based Hybridization Array System (CHAS) for Identification of Priority Enterohemorrhagic *E. coli* in Food
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.

**** For the detection of the priority VTEC serotypes, the CFIA and HC published method was used [26, 27, 28].