Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2010-2011 Targeted Surveys

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

Canada

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Executive Summary

The Food Safety Action Plan (FSAP) aims to modernize and enhance 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 responsible for 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. The presence of pathogens in leafy vegetables creates a potential risk for foodborne illness as leafy vegetables are often consumed raw. The disease causing bacterial pathogens *Escherichia coli* (*E. coli*) O157:H7 and *Salmonella* have accounted for the majority of the outbreaks associated with leafy vegetables.

Considering these factors 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 2010/11 survey were to generate baseline surveillance data on bacterial pathogens *Salmonella*, *Shigella*, *Campylobacter*, *Listeria monocytogenes*, *E. coli* O157 and other verotoxigenic *E. coli* (VTEC), as well as on the indicator of fecal contamination generic *E. coli*, for a variety of leafy vegetables available in the Canadian market. A total of 2596 leafy vegetable samples from various countries and production practices were collected and analyzed for one or more bacterial pathogen(s) or indicator of interest. The results indicate that bacterial pathogens were not detected in any of the leafy vegetable samples. Two samples were found to be unsatisfactory due to high levels of generic *E. coli* (> 1000 CFU/g). These two sample results triggered appropriate follow-up activities by the CFIA, though no recalls were required. In addition, elevated levels of generic *E. coli* (> 100 and ≤ 1000 CFU/g) were found in eight other samples. These samples were assessed as investigative and further evaluation resulted in no immediate follow-up activities. 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).

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. Moreover, 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's) Food Safety Action Plan (2) is one element of the government's broader FCSAP initiative. The goal of 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 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 (2010/11) represents part of the collection of over 10,000 leafy vegetable samples over five years (2008/09 – 2012/13) of microbiological targeted surveys, which was designed to gather baseline information on the occurrence of 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 FAO/WHO Codex Alimentarius Commission. Producers of fresh fruits and vegetables are encouraged to follow these 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 (GAP) and Good Manufacturing Practices (GMP) which, when applied, control and reduce the potential for contamination with microbial, chemical, and physical hazards at all stages of 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 sections of the FDA and FDR are enforced by the CFIA.

FSAP targeted surveys are primarily conducted for surveillance and not for regulatory compliance 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 March 2011, 61 foodborne disease outbreaks associated with leafy vegetables contaminated with bacterial pathogens were reported worldwide, with most of the reported cases occurring in North America and several cases occurred 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 this 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 2007 joint FAO/WHO Expert Meeting (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 FSAP for five years (2008/09 - 2012/13). The overall objective of this five-year study is to gather baseline information on the occurrence of various pathogens (bacterial,

viral and parasitic pathogens) of concern in leafy vegetables available to Canadians at retail.

This targeted survey (2010/11) 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, conventionally and/or organically produced leafy vegetables. During this targeted survey, a subset of samples was used as part of a pilot study to further evaluate the applicability of a method for the detection of Verotoxigenic *E. coli* (VTEC) in 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 consumption of contaminated food of animal origin (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 be contaminated with these bacterial pathogens in the field by improperly composted manure, contaminated water, wildlife feces, or poor hygienic practices of the farm workers (18).

Humans are the only host of the bacterial pathogen *Shigella* spp. 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, *Campylobacter* is also found naturally in the 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. and Canada (19), (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).

Verotoxigenic *E. coli* (VTEC), such as O157 and other non-O157 *E. coli* serogroups (e.g., O26, O103, O111, and O145) produce verocytotoxins that can cause human illness. 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) (17).

L. monocytogenes is widely distributed in the environment and is present 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* - 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 the 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 micro-organisms, 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 production and the time of sale.

2.3 Sample Collection

Leafy vegetable samples collected for this survey 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 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, as well as 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. Domestic samples were collected during the summer months (June-September). Imported samples were collected primarily in the fall, winter, and spring months. Samples that were labelled 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 is typical for surveys conducted at retail, and is also used by other federal partners such as the Public Health Agency of Canada (PHAC) for the retail component of their FoodNet Canada surveys (22). For samples tested for the VTEC pilot project, five sample units (n=5) from a single lot were collected to form a composite analytical sample.

Collected samples were required to be shipped under conditions that limited the growth of microorganisms during transit. Samples were declared “unfit” for analysis if there were issues regarding the conditions in which the sample was handled or shipped.

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 (Percentage) of Samples
Group I (organic leafy vegetables)	*E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes* (fresh-cut samples only), generic *E. coli*	Imported	Organic	581 (52.5%)
		Domestic	Organic	525 (47.5%)
		Subtotal		***1106 (100%)***
Group II (organic or conventional head lettuces)	*E. coli* O157, *Salmonella*, generic *E. coli*	Imported	Conventional or Organic	743 (57.6%)
		Domestic		547 (42.4%)
		Subtotal		***1290 (100%) (18 organic samples)***
Group III (organic or conventional leafy vegetables)	VTEC	Imported	Conventional or Organic	150 (75%)
		Domestic		50 (25%)
		Subtotal		***200 (100%) (1 organic sample)***

2.4.1 Sample Distribution by Country of Origin

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

Table 2 Imported Sample Distribution by Country of Origin

Country of Origin	Group I (organic leafy vegetables)	Group II (organic or conventional head lettuces)	Group III (organic or conventional leafy vegetables)
Chile	0	2	0
China	0	2	0
Costa Rica	0	1	0
Colombia	1	0	0
Dominican	1	0	2
Guatemala	0	5	0
Mexico	35	50	2
United States	539 (92.8%)	682 (91.8%)	145 (96.7%)
Unidentified	5	1	1
Total	581 (100%)	743 (100%)	150 (100%)

2.4.2 Sample Distribution by Product Type

The product types were tabulated for each leafy vegetable group (Table 3). A variety of lettuces accounted for approximately 44.5% and 44% of the group I and group III leafy vegetable samples respectively. A majority (94%) of the samples were lettuces in the head lettuce group (group II).

Table 3 Product Type in Each Group of Leafy Vegetable Samples

Product Type		Group I (organic leafy vegetables)	Group II (organic or conventional head lettuce)	Group III (organic or conventional leafy vegetables)
Arugula		70	0	5
Chicory		1	61	2
Collard		17	0	0
Dandelion		11	0	0
Kale		90	0	2
Salad mix		39 (23*)	0	35
Spinach		186	0	52
Spring mix		52 (26*)	0	7
Swiss chard		134	0	8
Watercress		2 (1*)	0	0
Others**		7	10	1
Boston Lettuce		0	168	1
Butter lettuce		1	59	2
Iceberg Lettuce		1	633	8
Head lettuce (not specified)		6	318	0
Romaine Lettuce		239	19	40
Leafy lettuce		206	19	28
Lettuce-mix		18	3	0
Lettuce-not specified		21	0	9
Lettuce	***Subtotal***	492 (44.5%)	1219 (94.0%)	88 (44.0%)
Total		**1106**	**1290**	**200**

* These fresh-cut samples were additionally tested for *L. monocytogenes*.

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

2.5 Methods Details

Samples were analysed mainly using the analytical methods published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods (23) (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, L. monocytogenes,* and VTEC, a two-step procedure was employed. Samples were first screened by polymerase chain reaction (PCR)-based methods. Presumptive positive results were confirmed by isolation, purification and identification procedures. For the detection of *Campylobacter,* fresh leafy vegetable samples were tested using a modified cultural method without the use of a PCR screening method. For the confirmation of the priority VTEC serotypes (O157, O26, O111, O103 and O145), the probe-based assay CHAS (cloth based hybridization array system) was used (24),(25). This method targets genes for key virulence factors and determinants specific to the five priority VTEC serotypes.

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

2.6 Assessment Guidelines

The assessment criteria used in this survey (Tables 4 and 5) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* (26) and associated methods published in Health Canada's *Compendium of Analytical Methods* (23), as well as Health Canada's "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (2004)" (updated in 2011) (21).

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 in this survey required some form of follow-up activity. This could include, for example, further sampling to verify the levels of generic *E. coli* in the samples in question.

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 Supplements 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
VTEC** (priority serotypes O157, O26, O111, O103 and O145) (CFIA and HC published method) ***	Absent in 125 g	Present in 125 g

* *Compendium of Analytical Methods* (23).

**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, the presence in foods is considered to be a violation of FDA Section 4(1)a and is therefore assessed by the CFIA as unsatisfactory.

*** Published methods (24), (25).

Table 5 Assessment Guidelines for Generic *E. coli* and *L. monocytogenes* in 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 & MFLP-74)	Absent in 25 g	Detected and ≤ 100 CFU/g	> 100 CFU/g

* Compendium of Analytical Methods (23)

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

*** Health Canada's "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods (2004)" (updated in 2011) (21)

2.7 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 Organic Leafy Vegetable Samples Analyzed for *E. coli* O157, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, and generic *E. coli*

In this group, a total of 1106 organic leafy vegetable samples, which included imported and domestically produced, whole and fresh-cut samples, were tested for pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, and *L. monocytogenes* (on 50 fresh-cut samples only), as well as indicator bacteria generic *E. coli*. No pathogens were found. A vast majority (99.2%) of the samples had no generic *E. coli* counts that exceeded 100 CFU/g and were assessed as satisfactory (Table 6). However, high levels of generic *E. coli* (>1000 CFU/g, Table 6 & 7) were found in two samples, which were assessed as unsatisfactory. Elevated levels of generic *E. coli* (>100 and ≤ 1000 CFU/g) were found in a total of seven samples (0.6%, 7/1106), including one fresh-cut sample (Table 6 & 8). These samples were assessed as investigative, as the *E. coli* counts were elevated but below the unsatisfactory threshold.

The CFIA conducted appropriate follow-up activities for the two unsatisfactory samples. No product recall resulted from the unsatisfactory sample. Further evaluation of the investigative samples (Table 8) resulted in no immediate follow-up sampling.

Table 6 Summary of the Results for Organic Leafy Vegetable Samples (whole and fresh-cut)

(All samples were analyzed for *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, *Campylobacter*, and generic *E. coli*. Some fresh-cut samples were also tested for *L. monocytogenes**)

Product Origin	Number of Samples	Assessment		
		Investigative	Unsatisfactory	Satisfactory
Imported	581 (45*)	0	0	581
Domestic	525 (5*)	7 (1*)	2	516
Total	**1106 (100%)**	**7 (0.6%)**	**2 (0.2%)**	**1097 (99.2%)**

Table 7 Summary of Unsatisfactory Samples

Product Origin	Product Type/Production Practice	Reason for Unsatisfactory Assessment
Domestic	Red chard/Organic	generic *E.coli*: >1000 CFU/g
	Red lettuce/Organic	generic *E.coli*: 1350 CFU/g

Table 8 Summary of Investigative Samples

Product Origin	Product Type/Production Practice	Generic *E. coli* Counts (CFU/g)
Domestic	Arugula/Organic	130
	Arugula/Organic	130
	Red leafy lettuce/Organic	190
	Arugula/Organic	270
	Wild water cress/Organic (*)	360
	Arugula/Organic	570
	Green leafy lettuce/Organic	800

* This sample was fresh-cut.

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

A combination of three bacterial organisms, *E. coli* O157:H7/NM, *Salmonella* and the indicator bacteria generic *E. coli*, were tested in organic or conventional head lettuce samples. Of the 1290 head lettuce samples analyzed, no pathogens were detected. No generic *E. coli* counts exceeded 100 CFU/g in the vast majority (99.9%) of the samples (Table 9). An elevated level of generic *E. coli* (160 CFU/g) was found in one sample (0.1%). This sample was assessed as investigative and further evaluation of the sample resulted in no immediate follow-up sampling.

Table 9 Summary of the Results for Head Lettuce Samples

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

Product Origin	Number of Samples	Assessment		
		Investigative	Unsatisfactory	Satisfactory
Imported	743	0	0	743
Domestic	547	1	0	546
Total	**1290 (100%)**	**1 (0.1%)**	**0 (0%)**	**1289 (99.9%)**

3.3 Leafy Vegetable Samples Analyzed for VTEC

As part of a pilot project designed to further evaluate the applicability of the VTEC methods (24, 25) to fresh fruit and vegetables and to collect information on the presence of VTEC in leafy vegetables, a total of 200 leafy vegetable samples were analyzed for VTEC.

No VTEC strains were detected in any of the samples analyzed.

Table 10 Summary of the Results for Leafy Vegetable Samples Analyzed for Verocytotoxin Genes VT1 & VT2

Product Category	Number of Samples	Assessment	
		Unsatisfactory	Satisfactory
Imported	150	0	150
Domestic	50	0	50
Total	**200 (100%)**	**0**	**200 (100%)**

3.4 Results Summary

The results of all testing are summarized in Table 11, according to the targeted microorganisms.

Table 11 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*	0/1324	2(8)/1072	2(8)/2396
***E. coli* O157/NM**	0/1324	0/1072	0/2396
Salmonella	0/1324	0/1072	0/2396
Shigella	0/581	0/525	0/1106
Campylobacter	0/581	0/525	0/1106
L. monocytogenes	0/45	0/5	0/50
VTEC	0/150	0/50	0/200

4 Discussion and Conclusion

The results of the 2010/11 survey indicate that no pathogens were detected in the leafy vegetable samples analyzed. Furthermore, a vast majority of the samples had acceptable levels of generic *E. coli*. Two leafy green vegetable samples were found to be unsatisfactory due to high levels of generic *E. coli* (> 1000 CFU/g). In addition, elevated levels of generic *E. coli* (> 100 and ≤ 1000 CFU/g) were found in another eight samples. These samples were assessed as investigative.

The CFIA followed up on the two unsatisfactory samples and no recalls resulted from the food safety investigations. Further evaluation of the samples assessed as investigative resulted in no immediate follow-up activities.

The overall finding of this survey suggests that the vast majority of fresh leafy vegetables in the Canadian market are produced and handled under acceptable GAPs and GMPs. The presence of high levels of generic *E. coli* in leafy vegetable samples occurs at a very low rate. Generic *E. coli* are not disease causing agents. However, their presence is used by the CFIA as an indicator that unwanted microorganisms may potentially be introduced during the production, processing, and marketing of these commodities.

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 oversights and promotes safe handling of foods throughout the food production chain. Surveillance activities will continue and the CFIA will inform stakeholders of its findings.

5 Acknowledgement

We would like to express our sincere thanks to Judy D. Greig, Laboratory for Foodborne Zoonoses, Public Health Agency Canada, for providing data on global foodborne disease outbreaks associated with leafy vegetables (Appendix B).

6 References

1. Government of Canada. *Food and Consumer Product Safety Action Plan [online]*. 2012. Accessed August 2013, http://www.tbs-sct.gc.ca/hidb-bdih/initiative-eng.aspx?Hi=85

2. Canadian Food Inspection Agency. *Food Safety Action Plan [online]*. 2012. Accessed August 2013, http://merlin/english/fssa/action/actione.asp

3. Canadian Food Inspection Agency. *Food Safety Science Committee Summary Report 2008 [online]*. 2008. Accessed October 2012, http://merlin.cfia-acia.inspection.gc.ca/english/fssa/invenq/guidoce.asp#refman5

4. CODEX Alimentarius committee in Food Hygiene. *The Code of Hygienic Practices for Fresh Fruits and Vegetables (CAC/RCP 53-2003) [online]*. 2011. Accessed August 2013, http://www.codexalimentarius.net/download/standards/10200/CXP_053e.pdf

5. CODEX Alimentarius committee in Food Hygiene. *Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969) [online]*. 2011. Accessed August 2013, http://www.codexalimentarius.net/download/standards/23/cxp_001e.pdf

6. Department of Justice Canada. *Food and Drugs Act [online]*. 2008. Accessed October 2012, http://laws-lois.justice.gc.ca/eng/acts/F-27/

7. Department of Justice Canada. *Food and Drug Regulations [online]*. 2012. Accessed October 2012, http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/index.html

8. Department of Justice Canada. *Fresh Fruit and Vegetable Regulations [online]*. 2011. Accessed October 2012, http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._285/index.html

9. Department of Justice Canada. *Canada Agricultural Products Act [online]*. 2005. Accessed August 2013, http://laws-lois.justice.gc.ca/eng/acts/C-0.4/

10. Kozak G. K., MacDonald D., Landry L. & Farber J. M. Foodborne Outbreaks in Canada Linked to Produce: 2001 through 2009 *J Food Prot* 2013; 76**,** 173-83.

11. Canadian Food Inspection Agency. *Advice to Consumers: Outbreak of E. Coli O157:H7 in the United State and Related Cases in Ontario*. 2008. Accessed 2013, http://epe.lac-bac.gc.ca/100/206/301/cfia-acia/2011-09-21/www.inspection.gc.ca/english/corpaffr/newcom/2008/20081004e.shtml

12. New Zealand Food Monitoring Program. *Food Safety and Hydroponiccally Grown Vegetables [online]*. 1999. Accessed 2013, www.foodsafety.govt.nz/elibrary/.../food_safety-project_examined.pdf

13. H. Davis, JP. Taylor, JN. Perdue, Jr. Stelma GN, Jr. Humphreys JM, 3rd. Rowntree R & KD. Greene. A Shigellosis Outbreak Traced to Commercially Distributed Shredded Lettuce *Am J Epidemiol.* 1988; 128**,** 1312-1321.

14. Oliveira M., Usall J., Solsona C., Alegre I., Vinas I. & Abadias M. Effects of Packaging Type and Storage Temperature on the Growth of Foodborne Pathogens on Shredded 'Romaine' Lettuce *Food Microbiol* 2010; 27**,** 375-80.

15. Farber J. M., Wang S. L., Cai Y. & Zhang S. Changes in Populations of *Listeria monocytogenes* Inoculated on Packaged Fresh-Cut Vegetables *J Food Prot* 1998; 61**,** 192-5.

16. WHO/FAO. *Microbiological Risk Assessment Series 14: Microbiological Hazards in Fresh Leafy Vegetables and Herbs [online]*. 2011. Accessed August 2013, ftp://ftp.fao.org/docrep/fao/011/i0452e/i0452e00.pdf

17. Food and Drug Administration. *Bad Bug Book*, 2012. Accessed June 2013, http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/

18. Centers for Disease Control and Prevention. Ongoing Multistate Outbreak of *Escherichia coli* Serotype O157:H7 Infections Associated with Consumption of Fresh Spinach--United States, September 2006 *MMWR Morb Mortal Wkly Rep.* 2006; 55**,** 1045-1046.

19. Painter J. A., Hoekstra R. M., Ayers T., Tauxe R. V., Braden C. R., Angulo F. J. & Griffin P. M. Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by Using Outbreak Data, United States, 1998-2008 *Emerg Infect Dis* 2013; 19**,** 407-15.

20. Public Health Agency of Canada. Estimate of Food-Borne Illness in Canada *[online]*. 2013. http://www.phac-aspc.gc.ca/efwd-emoha/efbi-emoa-eng.php

21. Health Canada. *Policy on Listeria monocytogenes in Ready-to-Eat Food [online]*. 2011. Accessed October 2012, http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2011-eng.php

22. PublicHealth Agency of Canada. *C-EnterNet* [online] 2013. Accessed October 2013, http://www.phac-aspc.gc.ca/c-enternet/index-eng.php

23. Health Canada. *Compendium of Analytical Methods [online]*. 2011. Accessed October 2012, http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php

24. Gill A, Martinez-Perez A., Mcilwham S. & Blais B.W. Development of a Method for the Detection of Verotoxin-Producing *E.coli* in Food *J of Food Protection* 2012; 75**,** 827-837.

25. Blais B. W. & Martinez-Perez A. A Simple Pcr-Based Macroarray System for Detection of Multiple Gene Markers in the Identification of Priority Enterohemorrhagic *Escherichia coli* *J Food Prot* 2011; 74**,** 365-72.

26. Health Canada. *Health Products and Food Branch Standards and Guidelines for the Microbiological Safety of Food - an Interpretive Summary [online]*. 2008. Accessed October 2012, http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume1/intsum-somexp-eng.php

Appendix A: List of Acronyms

CDC: Centers 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 Vegetables Contaminated with Bacterial Pathogens (1998 - 2011 March)

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	
15	2001	May	Infect. Dis. News Brief, 7 Sept 2001	Australia	Queensland	*Salmonella* Bovismorbificans	Lettuce, iceberg	41	

Case #	Year	Month	Source	Country	Province/ State	Microorganism	Vehicle	Number of Cases	Number of People Hospitalized (Deaths)
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	0

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	Nether-lands		*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	

Information in this appendix was prepared by Judy D. Greig, Laboratory for Foodborne Zoonoses , PHAC (Public Health Agency of Canada). The data presented were collected from several sources of information, such as peer-reviewed journals, newspapers, press releases, health units, national laboratory and government websites.

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

Bacterial Pathogens	Number of Outbreaks	Percentage of Outbreaks
E. coli O157	27	44.3
Other *E. coli*	5	8.2
Salmonella	19	31.1
Shigella	3	4.9
Campylobacter	3	4.9
Clostridium perfringens	2	3.3
Yersinia	2	3.3
Total	**61**	**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
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

* In *the Compendium of Analytical Methods* (23).

** MFLP-46 was performed as written with the following modifications. 25g from each sample were added to a filtered stomacher bag and stomached with 50 ml of peptone water for 2 min at 200 RPM. 25 mL of supernatant were removed and added to 100 mL of Park and Sanders Enrichment Broth, which is comprised of 100 mL of Brucella broth, 0.5 mL supplement A per 100 mL of broth, 0.5 mL supplement B per 100 mL of broth, 5 mL blood per 100 mL of broth. The sample was then incubated under microaerophilic atmosphere in a Tri-Gas incubator (5% O2, 10% CO2, 85% N2) at 37°C for 3 to 4 hours and then transferred to a 42°C incubator and incubated under microaerophilic atmosphere (as specified above) for 24 and 48 hours. Following incubation, the enrichment broth was plated as described in section 6.3 of MFLP-46.

*** 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.

**** Published methods (24, 25)