



Canadian Food
Inspection Agency

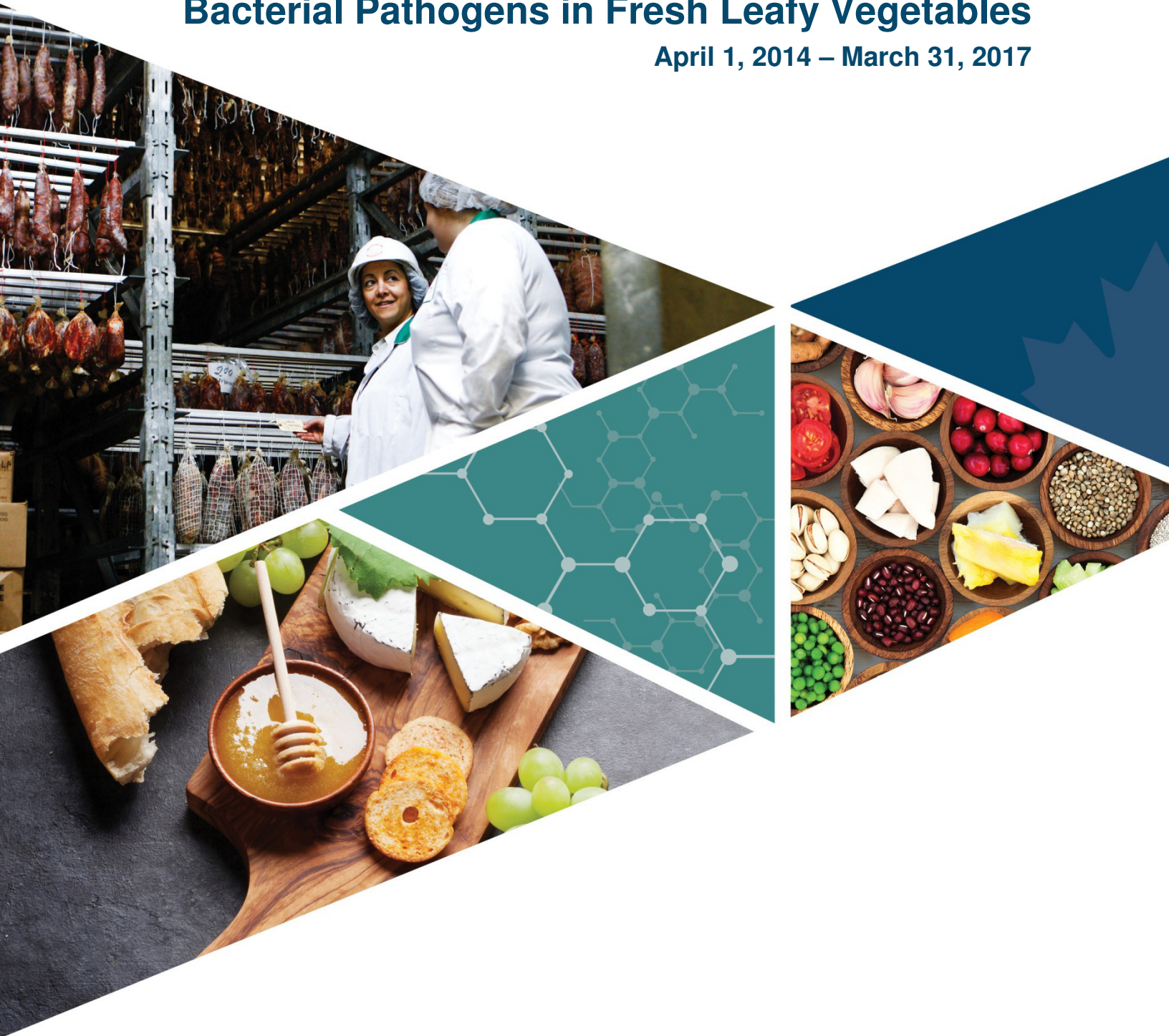
Agence canadienne
d'inspection des aliments

Food Microbiology – Targeted Surveys

FINAL REPORT

Bacterial Pathogens in Fresh Leafy Vegetables

April 1, 2014 – March 31, 2017



Summary

Fresh leafy vegetables are frequently consumed by Canadians in all age groups. Unfortunately, they have been associated with numerous outbreaks of foodborne illnesses worldwide. Fresh leafy vegetables can become contaminated with pathogens during production, harvest, post-harvest handling, packaging and distribution. Since fresh leafy vegetables are often consumed raw, the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, fresh leafy vegetables (leaf lettuce, spinach and arugula) were selected for targeted surveys. The purpose of the surveys was to generate baseline information on the occurrence of pathogenic bacteria in fresh leafy vegetables. Over the course of this study (April 1, 2014 – March 31, 2017), a total of 5508 samples were collected from retail locations in 11 cities across Canada. Five thousand thirty-eight (5038) samples were tested for *Salmonella* species (spp.) and *Shigella*. Four hundred seventy (470) samples were tested for non-O157 Verotoxigenic *Escherichia coli* (non-O157 VTEC). All 5508 samples were also tested for Verotoxigenic *Escherichia coli* O157 (*E.coli* O157) and generic *Escherichia coli* (*E.coli*). Generic *E.coli* is an indicator of the overall sanitation conditions throughout the food production chain.

Salmonella spp., *Shigella*, and *E.coli* O157 were not found in any samples. Non-O157 VTEC (O-untypable) was found in 1/470 (0.2%) samples. Elevated levels of generic *E.coli* ($100 < x \leq 1000$ Colony Forming Units (CFU)/g or Most Probable Number (MPN)/g) were found in 25/5508 (0.5%) samples and high levels of generic *E.coli* (>1000 CFU/g or MPN/g) were found in 7/5508 samples (0.1%).

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities such as additional sampling and facility inspections. Given the perishable nature of the products, the implicated products were no longer available on the market when the samples were declared as unsatisfactory and consequently no product recalls were issued. In addition, it was not possible to determine the source of the contamination, however corrective actions were implemented by the facilities.

Overall, our survey results suggest that almost all fresh leafy vegetables are safe for consumption. They can however be found to have elevated and high levels of generic *E.coli* and on rare occasions non-O157 VTEC. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

What Are Targeted Surveys?

Targeted surveys are used by the Canadian Food Inspection Agency (CFIA) to focus its surveillance activities on areas of highest health risk. The information gained from these surveys provides support for the allocation and prioritization of the Agency's activities to areas of greater concern. Originally started as a project under the Food Safety Action Plan (FSAP), targeted surveys have been embedded in the CFIA's regular surveillance activities since 2013. Targeted surveys are a valuable tool for generating information on certain hazards in foods, identifying and characterizing new and emerging hazards, informing trend analysis, prompting and refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

Food safety is a shared responsibility. The Canadian Food Inspection Agency works with federal, provincial, territorial and municipal governments and provides regulatory oversight of the food industry to promote safe handling of foods throughout the food production chain. The food industry and retail sectors in Canada are 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.

Why Did We Conduct This Survey?

Fresh leafy vegetables are frequently consumed by Canadians in all age groups¹. Unfortunately, they have been associated with numerous recalls and outbreaks of foodborne illnesses worldwide^{2, 3}. In 2007 a joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Committee classified fresh leafy green vegetables as the highest priority group of concern among fresh fruits and vegetables in terms of microbial hazards⁴.

Fresh leafy vegetables can become contaminated with pathogens during production, harvest, post-harvest handling, packaging and distribution. Since fresh leafy vegetables are often consumed raw, the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, fresh leafy vegetables were selected for targeted surveys in two phases with phase one covering the period from April 1, 2009 to March 31, 2014 and phase two covering the period from April 1, 2014 to March 31, 2017. The second phase of the survey focused on specific types of leafy vegetables that were found to be more often associated with contamination in phase one (i.e.,

leaf lettuce, spinach and arugula). This survey was implemented to gain further information on the occurrence of *Salmonella* species (spp.), *Shigella*, Verotoxigenic *Escherichia coli* O157 (*E. coli* O157), non-O157 Verotoxigenic *Escherichia coli* (non-O157 VTEC) and generic *Escherichia coli* (*E.coli*) in these product types at retail in Canada. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food production chain. This report details results from phase two of the survey. Results from phase one of the survey are detailed in four separate reports ([2009-2010](#), [2010-2011](#), [2011-2012](#), [2012-2014](#)).

What Did We Sample?

For this survey, a sample consisted of a single unit (e.g., individual consumer-size package(s) or bulk from a single lot) with a total weight of at least 250g. All samples were collected from national retail chains and local/regional grocery stores located in 11 major cities across Canada. These cities encompassed four geographical areas: Atlantic (Halifax and Saint John), Quebec (Quebec City, Montreal), Ontario (Toronto, Ottawa), and the West (Vancouver, Kelowna, Calgary, Saskatoon and Winnipeg). The number of samples collected from these cities was in proportion to the relative population of the respective areas. Samples were collected between April 1, 2014 and March 31, 2017.

A variety of whole or trimmed, pre-packaged or bulk fresh leafy vegetables (leaf lettuce, spinach and arugula) were sampled. Samples were domestic or imported, organic or conventionally grown. Contrary to the sampling design of phase one where imported samples were not collected in the summer, phase two of this survey was designed so that imported samples would be collected throughout the year. Phase two was also designed so that the ratio of domestic to imported samples was 1:2 and the ratio of organic to conventional samples was 1:1. Similar to phase one, phase two domestic sampling was focused in the summer.

What Analytical Methods Were Used and How Were Samples Assessed?

Samples were analyzed using methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*⁵ (Table 1). The assessment criteria used in this survey (Table 1) are based on the principles of Health Canada's *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*⁶.

Table 1 - Analytical Methods and Assessment Criteria for Bacteria in Fresh Leafy Vegetables

Bacterial Analysis	Method Identification Number*	Assessment Criteria		
		Satisfactory	Investigative	Unsatisfactory
<i>Salmonella</i> spp.	MFHPB-20 MFLP-38 MFLP-29	Absent in 25g	Not Applicable (N/A)	Present in 25g
<i>Shigella</i>	MFLP-25 MFLP-26	Absent in 25g	N/A	Present in 25g
<i>E. coli</i> O157	MFLP-30 MFLP-80 MFLP-52	Absent in 25g	N/A	Present in 25g
Non-O157 VTEC	MFLP-52	Absent in 25 g	Non-O157 VTEC present in 25 g	N/A
Generic <i>E. coli</i>	MFHPB-19 MFHPB-27	≤ 100 MPN/g or CFU/g	100 < x ≤ 1000 MPN/g or CFU/g	> 1000 MPN/g or CFU/g

* The methods used were the published versions at the time of analysis

No assessment guidelines had been established in Canada for the presence of *Salmonella* spp., *Shigella* or non-O157 VTEC in fresh leafy vegetables at the time of writing this report. As *Salmonella* spp., and *Shigella* are considered pathogenic to humans their presence was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)a⁸ and therefore in the absence of assessment guidelines was assessed by the CFIA as unsatisfactory. The detection of non-O157 VTEC was assessed as investigative, indicating that further follow-up actions may be warranted depending upon the serotype identified. All non-O157 VTEC have the potential to cause human illnesses, however at this time there is no established virulence profile for pathogenic non-O157 VTEC⁷ and consequently unless previously associated with human illness it is difficult to establish its human health significance. Of the non-O157 VTEC serotypes identified to date, the following have been identified as causing the majority of non-O157 VTEC infections in Canada (O26, O103, O111, O117, O121, and O145)⁷.

Unlike harmful bacterial pathogens (e.g. *Salmonella*, *E. coli* O157), generic *E. coli* is commonly found in the intestines of humans and most strains are harmless. It is considered to be an indicator organism and levels of generic *E. coli* found in a food product are used to assess the overall sanitation conditions throughout the food chain from production to the point of sale. Its presence at some levels is tolerated on agricultural products. An investigative assessment which may result in further follow-up actions is associated with elevated levels of generic *E. coli* (100 < x ≤ 1000 Most Probable Number (MPN)/g or Colony Forming Units (CFU)/g). As the

results are based on the analysis of one unit (n=1), further sampling is required to verify the levels of generic *E. coli* of the lot. An unsatisfactory assessment is associated with high levels of generic *E.coli* (> 1000 MPN/g or CFU/g) as it may indicate a breakdown in Good Agricultural Practices, and therefore possibly warranting the initiation of follow-up activities to determine the source of the contamination and improve sanitation conditions along the food chain.

What Were The Survey Results?

Over the course of this study (April 1, 2014 to March 31, 2017), a total of 5508 samples were collected and tested for bacterial pathogens of concern as well as generic *E. coli*. Five thousand thirty-eight (5038) samples were tested for *Salmonella* spp. and *Shigella* (Group I) throughout the entire survey period. Four hundred seventy samples (470) were tested for non-O157 VTEC (Group II) during the first year of the survey only. All 5508 samples (Group I and II) were also tested for *E. coli* O157 and generic *E. coli* throughout the entire survey period.

Salmonella spp., *Shigella*, and *E. coli* O157 were not found in any samples. A non-O157 VTEC (O-untypeable) was found in 1/470 (0.2%) samples. Elevated levels of generic *E.coli* (100<x≤1000 MPN/g or CFU/g) were found in 25/5508 (0.5%) samples and high levels of generic *E.coli* (>1000 MPN/g or CFU/g) were found in 7/5508 samples (0.1%). Sample assessment results can be found in Table 2.

Table 2 - Assessment Results of Fresh Leafy Vegetable Samples

Sampling Group	Bacterial Analysis	Number of Samples Tested	Satisfactory	Investigative	Unsatisfactory
I	<i>Salmonella</i> spp.	5038	5007	N/A	0
	<i>Shigella</i>			N/A	0
	<i>E. coli</i> O157			N/A	0
	Generic <i>E.coli</i>			24	7
II	Non-O157 VTEC	470	468	1*	0
	<i>E. coli</i> O157			N/A	0
	Generic <i>E. coli</i>			1	0
Total		5508	5475	26	7

*O-untypeable

Of the 5508 samples tested, 2981 (54%) were conventional and 2527 (46%) were organic (Table 3).

Table 3 – Assessment Results of Fresh Leafy Vegetables by Production Practice

Production Practice	Number of Samples Tested (% of Total Samples)	Satisfactory	Investigative		Unsatisfactory Generic <i>E. coli</i> (> 1000 MPN/g or CFU/g)
			Non-O157 VTEC*	Generic <i>E. coli</i> (100 < x ≤ 1000 MPN/g or CFU/g)	
Conventional	2981 (54%)	2965	1	15	0
Organic	2527 (46%)	2510	0	10	7
Total	5508	5475	1	25	7

*non-O157 VTEC tested in 470 samples

Of the 5508 samples tested, 2195 (40%) were domestic and 3313 (60%) were imported (Table 4).

Table 4 – Assessment Results of Fresh Leafy Vegetables by Country of Origin

Product Origin	Number of Samples Tested (% of Total Samples)	Satisfactory	Investigative		Unsatisfactory Generic <i>E. coli</i> (> 1000 MPN/g or CFU/g)
			Non-O157 VTEC*	Generic <i>E. coli</i> (100 < x ≤ 1000 MPN/g or CFU/g)	
Domestic	2195 (40%)	2177	1	15	2
Import	3313 (60%)	3298	0	10	5
<i>United States</i>	3051 (55%)	3038	0	9	4
<i>Mexico</i>	46 (0.8%)	46	0	0	0
<i>USA & Mexico</i>	199 (3.6%)	197	0	1	1
<i>Imported (Unknown Country)</i>	15 (0.3%)	15	0	0	0
<i>France</i>	2 (<0.1%)	2	0	0	0
Total	5508	5475	1	25	7

*non-O157 VTEC tested in 470 samples

Domestic sampling was focused in the summer and fall seasons (Table 5) while sampling of imported produce was evenly distributed throughout the year (Table 6).

Table 5 - Assessment Results of Domestic Fresh Leafy Vegetable Samples by Season Sampled

Season Sampled	Number of Samples Tested (% of Total Samples)	Satisfactory	Investigative		Unsatisfactory Generic <i>E.coli</i> (> 1000 MPN/g or CFU/g)
			Non-O157 VTEC*	Generic <i>E.coli</i> (100 < x ≤ 1000 MPN/g or CFU/g)	
Winter and Spring (December 1 – May 31)	3 (<0.1%)	3	0	0	0
Summer (June 1 – August 31)	1413 (64%)	1402	1	10	0
Fall (September 1 – November 30)	779 (35%)	772	0	5	2
Total	2195	2177	1	15	2

*non-O157 VTEC tested in 470 samples of which 366 were domestic

Table 6 - Assessment Results of Imported Fresh Leafy Vegetable Samples by Season Sampled

Season Sampled	Number of Samples Tested (% of Total Samples)	Satisfactory*	Investigative Generic <i>E.coli</i> (100 < x ≤ 1000 MPN/g or CFU/g)	Unsatisfactory Generic <i>E.coli</i> (> 1000 MPN/g or CFU/g)
Winter (December 1 – February 28)	812 (25%)	812	0	0
Spring (March 1 – May 31)	773 (23%)	772	1	0
Summer (June 1 – August 31)	861 (26%)	856	4	1
Fall (September 1 – November 31)	867 (26%)	858	5	4
Total	3313	3298	10	5

*non-O157 VTEC tested in 470 samples of which 104 were imported

A focused variety of fresh leafy vegetable product types were analysed (Table 7).

Table 7 – Assessment Results of Fresh Leafy Vegetable Samples by Product Type

Product Type	Number of Samples Analysed (% of Total Samples)	Satisfactory	Investigative		Unsatisfactory
			Non-0157 VTEC*	Generic <i>E. coli</i> (100 < x ≤ 1000 MPN/g or CFU/g)	Generic <i>E. coli</i> (> 1000 MPN/g or CFU/g)
Arugula	620 (11%)	615	0	4	1
Leaf Lettuce	2792 (51%)	2781	1	8	2
Spinach	1979 (36%)	1963	0	12	4
Mixed Greens	117 (2%)	116	0	1	0
Total	5508	5475	1	25	7

*non-O157 VTEC tested in 470 samples

Further details about each investigative and unsatisfactory sample are provided in Table 8.

Table 8 – Detailed Information About Investigative and Unsatisfactory Samples

Product Type	Investigative	Unsatisfactory
Arugula	Trimmed, Pre-Packaged, Pre-Washed Organic Baby Arugula from the United States (2) Trimmed, Pre-Packed, Pre-Washed Conventional Arugula from Canada (1) Trimmed, Pre-Packaged, Pre-Washed Conventional Baby Arugula from the United States (1)	Trimmed, Pre-Packaged, Pre-Washed, Organic Baby Arugula from the United States and Mexico (1)
Leaf Lettuce	Whole, Bulk, Conventional Red Leaf Lettuce from Canada (1)* Whole, Bulk, Organic Romaine from Canada (1) Whole, Bulk, Conventional Romaine from Canada (3) Whole, Bulk, Conventional Green Leaf Lettuce from Canada (2) Whole, Bulk, Conventional Red Leaf Lettuce from Canada (2)	Whole, Bulk, Organic Romaine from Canada (1) Whole, Bulk, Organic Romaine from United States (1)
Spinach	Trimmed, Pre-Packaged, Pre-Washed, Organic Baby Spinach from United States (5) Trimmed, Pre-Packaged, Pre-Washed, Organic Baby Spinach from United States and Mexico (1) Trimmed, Bulk, Organic Spinach from Canada (1) Trimmed, Pre-Packaged, Pre-Washed, Conventional Baby Spinach from Canada (1) Trimmed, Bulk, Conventional Baby Spinach from Canada (2) Whole, Bulk, Conventional Spinach from Canada (1) Trimmed, Pre-Packaged, Pre-Washed Conventional Spinach from United States (1)	Trimmed, Pre-Packaged, Pre-Washed, Organic Baby Spinach from United States (3) Trimmed, Bulk, Organic Baby Spinach from Canada (1)
Mixed Greens	Trimmed, Pre-Packaged, Conventional Leaf Lettuce from Canada (1)	-

*Investigative for non-O157 VTEC

What Do The Survey Results Mean?

In this survey, all (100%) of the fresh leafy vegetable (leaf lettuce, spinach and arugula) samples analyzed were free of *Salmonella* spp., *Shigella*, and *E. coli* O157. A non-O157 VTEC (O-untypable) was found in 1/470 (0.2%) samples. Elevated levels of generic *E. coli* ($100 < x \leq 1000$ MPN/g or CFU/g) were found in 25/5508 (0.5%) samples and high levels of generic *E. coli* (>1000 MPN/g or CFU/g) were found in 7/5508 samples (0.1%).

Results similar to ours were found in studies conducted in Germany⁹, the US¹⁰ and Canada¹¹. The 2015 German study⁹ investigated the microbiological quality of fresh produce collected from retail stores in Northern Germany and found no presence of *E. coli* O157, non-O157 VTEC or *Salmonella* spp. in any of the leaf lettuce samples tested (n=40). The 2009-2010 US study¹⁰ investigated the microbiological quality of fresh produce at retail stores located in Maryland, USA and found no presence of *Salmonella* spp in any of the spinach samples tested (n=69). Of the 69 spinach samples tested, *E. coli* O157 was detected in one sample and generic *E. coli* was detected at levels ranging from <1.0 log CFU/g (limit of detection) to 1.78 log CFU/g (median = <1 log CFU/g). The 2012 Canadian study¹¹ investigated the microbiological quality of locally grown leaf lettuce samples sold at Farmers' Markets in Vancouver, British Columbia and found no presence of *E. coli* O157 or non-O157 VTEC in any of the leaf lettuce samples tested (green (n=27), red (n=24) and romaine (n=17)). Generic *E. coli* was detected in 13% (9/68) of the leafy lettuce samples at levels ranging from undetectable to 3 log CFU/g (mean=0.8 log CFU/g, median=0.7 log CFU/g). Any variations observed in prevalence rates between studies may be due to reasons such as differences in sanitation practices, product types tested, analytical methods, sample size, study design etc.

No trends were observed in our study with respect to production practice (Table 3), country of origin (domestic vs. imported) (Table 4) or product type (Table 7). A 2011¹² Canadian study also found no significant difference in the prevalence of generic *E. coli* between conventional (n=18) and organic (n=7) leafy green vegetable products. The 2012 Canadian¹¹ study mentioned previously also found no significant difference in the prevalence of generic *E. coli* between the leaf lettuce product types tested (green (n=27), red (n=24) and romaine (n=17)). The imported samples in our study showed a higher percentage of investigative and unsatisfactory samples in the summer and the early-mid fall seasons as compared to the winter and spring seasons (Table 6) which is not unusual as bacteria could multiply faster in warmer weather.

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities such as additional sampling and facility inspections. Given the perishable nature of the products, the

implicated products were no longer available on the market when the samples were declared as unsatisfactory and consequently no product recalls were issued. In addition, it was not possible to determine the source of the contamination, however corrective actions were implemented by the facilities.

Overall, our survey results suggest that almost all fresh leafy vegetables are safe for consumption. They can however be found to have elevated and high levels of generic *E.coli* and on rare occasions non-O157 VTEC. Consequently, as with all foods safe handling practices are recommended for producers, retailers and consumers.

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