

Food Microbiology – Targeted Surveys FINAL REPORT

Bacterial Pathogens in Fresh Leafy Herbs

April 1, 2014 - March 31, 2016



Summary

Fresh leafy herbs are frequently consumed by Canadians in all age groups. Unfortunately, fresh leafy herbs have been associated with numerous outbreaks of foodborne illnesses worldwide. Fresh leafy herbs can become contaminated with pathogens during production, harvest, post-harvest handling, processing, packaging and distribution. Since fresh leafy herbs 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 herbs were selected for targeted surveys. The purpose of targeted surveys is to generate baseline information on the occurrence and distribution of pathogenic bacteria in food. Over the course of this study (April 1, 2014 – March 31, 2016), a total of 2957 samples were collected from retail locations in 11 cities across Canada and tested for bacterial pathogens of concern (*Salmonella* species (spp.), *Shigella* and *Escherichia coli* O157:H7 (*E.coli* O157:H7)) as well as generic *E. coli*. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food production chain.

In this survey, over 99% of the fresh leafy herb samples analyzed were free of pathogenic bacteria tested for. *Shigella and E.coli* O157:H7 were not found in any samples. *Salmonella* spp. was found in 2 (<0.1%) samples, elevated levels of generic *E.coli* (100<x≤1000 MPN/g) were found in 20 samples (0.7%) and high levels of generic *E.coli* (>1000 MPN/g) were found in 11 samples (0.4%).

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities such as facility inspections and additional sampling. Both *Salmonella* spp. positive samples resulted in recalls of the affected products. In cases where high levels of generic *E.coli* were found, no direct product action was possible. This was due to the fact that the implicated products were no longer available on the market when the sample was declared as unsatisfactory given the perishable nature of the products. In addition, in most cases it was not possible to determine the source of contamination however corrective actions were implemented by the processing facilities. There were no reported illnesses linked to the *Salmonella* contaminated products.

Overall, our survey results suggest that almost all fresh leafy herbs are safe for consumption however they can sporadically become contaminated with *Salmonella* spp. Consequently, 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 herbs are frequently consumed by Canadians in all age groups¹. Unfortunately, fresh leafy herbs have been associated with numerous recalls and outbreaks of foodborne illnesses worldwide and in particular those related to *Salmonella*² spp. 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 (including leafy green herbs) as the highest priority group of concern among fresh fruits and vegetables in terms of microbial hazards³.

Fresh leafy herbs can become contaminated with pathogens during production, harvest, postharvest handling, processing, packaging and distribution. Since fresh leafy herbs are delicate they may not be subject to as intense a washing step as other leafy greens and also given that they 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 herbs were selected for targeted surveys in two phases with Phase 1 covering the period from 2009/10 - 2013/14 and Phase 2 covering the period from 2014/15-2015/16. The purpose was

to gather baseline information on the occurrence and distribution of *Salmonella* spp., *Shigella*, *Escherichia coli* O157:H7 (*E. coli* O157:H7) and generic *E. coli* in this commodity 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 2 of the survey from April 1, 2014 to March 31, 2016. Results from Phase 1 of the survey showed similar incidences of *Salmonella* spp. and generic *E. coli* as compared to those reported here and are detailed in <u>separate</u> reports.

What Did We Sample?

For this survey, a sample consisted of a single unit (e.g., individual consumer-size package(s) from a single lot) with a total weight of at least 150 g. 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, 2016.

A variety of domestic, imported, organic and conventional fresh leafy herbs were sampled. Contrary to the sampling design of Phase 1 where imported samples were not collected in the summer, Phase 2 of this survey was designed so that imported samples would be collected throughout the year and in a ratio of imported to domestic samples of 2:1. Additionally, Phase 2 sampling involved an increase in the sampling of organic samples from Phase 1 so that the ratio of organic to conventional samples was 1:1, as well as an increased focus on product types that were identified in Phase 1 to more likely be contaminated.

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*⁴ (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*⁵.

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

 Herbs

Bacterial	Method Identification	Assessment Criteria				
Analysis	Number*	Satisfactory	Investigative	Unsatisfactory		
Salmonella spp.	MFHPB-20	Absent in 25g	Not Applicable (N/A)	Present in 25g		
Shigella	MFLP-25	Absent in 25g	N/A	Present in 25g		
<i>E. coli</i> O157:H7	MFLP-30, MFHPB-10	Absent in 25g	N/A	Present in 25g		
Generic E. coli	MFHPB-19 MFHPB-27	<u><</u> 100 MPN/g or CFU/g	100 < x <u><</u> 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. or *Shigella* in fresh leafy herbs at the time of writing this report. However, these microorganisms are considered pathogenic to humans and as such in the absence of assessment guidelines, their presence in fresh leafy herbs is considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1) and is therefore assessed by the CFIA as unsatisfactory.

Unlike harmful bacterial pathogens (e.g. *Salmonella, E. coli* O157:H7), 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* ($100 < x \le 1000 \text{ MPN/g}$). 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) as it may indicate a breakdown in Good Agricultural Practices, or Good Manufacturing Practices (sanitation practices), and therefore possibly warranting the initiation of follow-up activities to, for example, improve sanitation conditions along the food chain.

What Were The Survey Results?

Over the course of this study (April 1, 2014 to March 31, 2016), a total of 2957 samples were tested for bacterial pathogens of concern (*Salmonella* spp., *Shigella, E. coli* O157:H7) as well as generic *E. coli*. Generic *E. coli* is an indicator of the overall sanitation conditions throughout the food production chain. Sample assessment results can be found in Table 2.

	Assessment Results			
Bacterial Analysis	Satisfactory (% of total samples)	Investigative (% of total samples)	Unsatisfactory (% of total samples)	
Salmonella		N/A	2 (<0.1%)	
Shigella	2924 (98.9%)	N/A	0	
<i>E. coli</i> O157:H7		N/A	0	
Generic E. coli		20 (0.7%)	11 (0.4%)	
Total	2924	20	13	

Table 2 - Assessment Results of Fresh Leafy Herb Samples

Shigella and E.coli O157:H7 were not found in any samples, Salmonella spp. was found in 2 (<0.1%) samples, 20 samples (0.7%) were found to have elevated levels of generic E.coli ($100 < x \le 1000 \text{ MPN/g}$) and 11 samples (0.4%) were found to have high levels of generic E.coli (>1000 MPN/g). The Salmonella spp. samples were identified as serotypes Pomona and Thompson.

Of the 2957 samples tested, 1546 (52%) were conventional, 1411 (48%) were organic (Table 3).

Table 3 - Assessment	Results of Fresh	Leafy Herbs b	v Production Practice
		Ecury nerbs b	y i roudollori i ruolloc

Production Practice	Number of		Investigative	Unsatisfactory	
	Samples Tested (% of Total Samples)	Satisfactory	sfactory Generic <i>E.coli</i> (100 < x ≤ 1000 MPN/g)	Generic <i>E.coli</i> (> 1000 MPN/g)	<i>Salmonella</i> spp.
Conventional	1546 (52%)	1529	11	4	2
Organic	1411 (48%)	1395	9	7	0

Of the 2957 samples tested, 1004 (34%) were domestic, 1952 (66%) were imported and 1 (<0.1%) was of unknown origin (Table 4).

	Number of		Investigative Generic <i>E.coli</i> (100 < x ≤ 1000 MPN/g)	Unsatisfactory	
Product Origin	Samples Tested (% of Total Samples)	Satisfactory		Generic <i>E.coli</i> (> 1000 MPN/g)	Salmonella spp.
Domestic	1006 (34%)	996	8	2	0
United States	1558 (53%)	1548	7	3	0
Mexico	195 (7%)	192	1	2	0
Colombia	72 (2%)	67	3	2	0
Israel	42 (1%)	42	0	0	0
Dominican Republic	39 (1%)	39	0	0	0
Imported (Unknown)	13 (0.4%)	13	0	0	0
Morocco	11 (0.4%)	11	0	0	0
Costa Rica	6 (0.2%)	5	0	1	0
USA & Mexico	6 (0.2%)	6	0	0	0
Vietnam	5 (0.2%)	2	1	0	2*
Peru	2 (<0.1%)	2	0	0	0
Thailand	1 (<0.1%)	1	0	0	0
Unknown – Packaged in Canada	1 (<0.1%)	0	0	1	0
Total	2957	2924	20	11	2

Table 4 - Assessment Results of Fresh Leafy Herbs by Country of Origin

*2 samples were positive for *Salmonella* spp. and elevated levels of generic *E.coli* ($100 < x \le 1000$ MPN/g).

Samples were taken throughout the year and details can be found in Table 5.

Table 5 - Assessment Results of Fresh Leaf	y Herb Samples by	y Season Samp	led
--	-------------------	---------------	-----

Number of		Investigative	Unsatisfactory		
Season Sampled	Samples Tested (% of Total Samples)	Satisfactory	Generic <i>E.coli</i> (100 < x <u><</u> 1000 MPN/g)	Generic <i>E.coli</i> (> 1000 MPN/g)	Salmonella spp.
Winter	455 (15%)	453	1	1	0
Spring	420 (14%)	419	0	1	0
Summer	1040 (35%)	1032	4	4	0
Fall	1042 (35%)	1020	15	5	2

A variety of fresh leafy herb product types were analysed and are detailed in Table 6.

	Number of		Investigative	Unsatisfactory	
Product Type	Samples Analysed (% of Total Samples)	Satisfactory	Generic <i>E.coli</i> (100 < x <u><</u> 1000 MPN/g)	Generic <i>E.coli</i> (>1000 MPN/g)	<i>Salmonella</i> spp.
Parsley	1411 (48%)	1400	8	3	0
Cilantro	635 (22%)	632	3	0	0
Dill	351 (12%)	348	0	3	0
Basil	200 (7%)	194	3	3	0
Mint*	155 (5%)	150	3	2	2
Rosemary	56 (2%)	55	0	1	0
Sage	37 (10%)	36	1	0	0
Thyme	37 (1%)	36	1	0	0
Chives	27 (1%)	26	1	0	0
Oregano	22 (0.7%)	22	0	0	0
Tarragon	14 (<1%)	14	0	0	0
Savory	8 (<1%)	8	0	0	0
Marjoram	4 (<1%)	3	0	1	0
Total	2957	2924	20	11	2

Table 6 - Assessment Results of Fresh Leafy Herb Samples by Product Type

*2 samples were positive for *Salmonella* spp. and elevated levels of generic *E.coli* ($100 < x \le 1000$ MPN/g).

What Do The Survey Results Mean?

In this survey, over 99% of the fresh leafy herb samples analyzed were free of pathogenic bacteria tested for. *Shigella* and *E.coli* O157 were not found in any samples while *Salmonella* spp. was found in two samples (<0.1%). Elevated levels of generic *E.coli* (100 < $x \le 1000$ MPN/g) were found in 20 samples (0.7%) and high levels of generic *E.coli* (>1000 MPN/g) were found in 11 (0.4%) samples.

The *Salmonella* spp. found in our survey were identified as serotypes: Pomona and Thompson. *Salmonella* Thompson was identified as the cause of a 1999 US outbreak⁶ involving 76 cases where the implicated product was fresh cilantro. *Salmonella* Pomona is a highly pathogenic serotype that has been identified as the cause of numerous outbreaks⁷ in the US where the implicated source was human contact with turtles and reptiles.

The prevalence of *Salmonella* spp. (<0.1%), elevated levels of generic *E.coli* (100 < x \leq 1000 MPN/g) (0.7%) and high levels of generic E.coli (>1000 MPN/g) (0.4%) in our survey were lower than two studies conducted in 2007⁸ and 2014⁹ in the UK. The UK studies investigated the microbiological quality of fresh leafy herbs at retail. The 2007⁸ UK study showed the following prevalence rates: *Salmonella* spp. 0.5% (18/3760), generic *E.coli* (100 < x \leq 1000 MPN/g) 2.5%

(95/3760) and generic *E.coli* (>1000 CFU/g) 1.1% (42/3760). The 2014⁹ UK study showed the following prevalence rates: *Salmonella* spp. 1.2% (9/774) and generic *E.coli* (>100 CFU/g) 11% (88/774). A 2011¹⁰ Canadian study found generic *E.coli* in 6.6% (4/61) of the samples tested and did not detect any samples with *E.coli* O157 (0/61) or *Salmonella* spp. (0/61). The varying prevalence rates between studies may be due to several reasons such as differences in sanitation practices, package types (the 2007⁸ UK included pre-cut, pre-packaged, open-cut bunches, herbs grown in pots), product types tested, methodology, study design etc.

No trends were observed with respect to production practice (Table 3). The 2011^{10} Canadian study also concluded that there was no significant difference in the prevalence of *E.coli* between conventional and organic product. With respect to country of origin, both *Salmonella* spp. positive samples were mint produced in Vietnam (Table 4). In addition, 60% of the samples with elevated ($100 < x \le 1000$ MPN/g) levels of generic *E.coli* and greater than 80% of the samples with high (>1000 MPN/g) levels of generic *E.coli* were imported (Table 4). Our study found higher rates of investigative and unsatisfactory samples in the fall and summer seasons (Table 5) which is not unusual as bacteria multiply faster in warmer weather.

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities such as facility inspections and additional sampling. Both *Salmonella* spp. positive samples resulted in recalls of affected products. In cases where high levels of generic *E.coli* were found, no direct product action was possible. This was due to the fact that the implicated products were no longer available on the market when the sample was declared as unsatisfactory given the perishable nature of the products. In addition, in most cases it was not possible to determine the source of contamination however corrective actions were implemented by the processing facilities. There were no reported illnesses linked to the *Salmonella* contaminated products.

Overall, our survey results suggest that almost all fresh leafy herbs are safe for consumption however they can sporadically become contaminated with *Salmonella* spp. Consequently, safe handling practices are recommended for producers, retailers and consumers.

References

1. PHAC, Foodbook Report, PHAC, Editor. 2015.

2. Zweifel, C. and R. Stephan, *Spices and herbs as source of Salmonella-related foodborne diseases.* Food Research International, 2012. **45**: p. 765-769.

3. FAO/WHO. *Microbiological Hazards in Fresh Fruits and Vegetables*. 2008; Available from: <u>http://www.fao.org/fileadmin/templates/agns/pdf/jemra/FFV_2007_Final.pdf</u>.

4. HealthCanada, Compendium of Analytical Methods, H. Canada, Editor.

5. HealthCanada, Health Products and Food Brnach (HPFB) Standards and Guidelines for Microbiological Safety of Food - An Interpretive Summary, H. Canada, Editor. 2008.

6. Campbell, J.V., et al., *An Outbreak of Salmonella Serotype Thompson Associated with Fresh Cilantro.* The Journal of Infectious Diseases, 2001. **2001**(183): p. 984-7.

7. Gong, S., et al., *Highly pathogenic Salmonella Pomona was first isolated from the exotic re-eared slider* (*Trachemys scripta elegans*) in the wild in China: Implications for public health. Science of the Total Environment, 2014. **468-469**(January 2014): p. 28-30.

8. Elviss, N.C., et al., *Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis.* International Journal of Food Microbiology, 2009. **134**(2009): p. 83-88.

9. Willis, C., et al., *As assessment of the microbiological safety of fresh whole-leaf herbs from retail premises in the United Kingdom with a focus on Salmonella spp.* Journal of Applied Microbiology, 2015. **119**: p. 827-833.

10. Allen, K.J., et al., *Microbiological survey of imported produce available at retail across Canada.* International Journal of Food Microbiology, 2011. **162**(2013): p. 135-142.