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 Fresh Leafy Herbs

RDIMS # 6205208

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 herbs have been reported to be responsible for numerous outbreaks of foodborne illness worldwide. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has ranked leafy herbs as the highest priority of concern among fresh fruits and vegetables in terms of microbiological hazards. Leafy herbs can become contaminated with various foodborne pathogens in the field by animals, improperly composted manure, and contaminated irrigation water during primary production. Leafy herbs can also become contaminated during harvest, post-harvest handling, packaging and distribution by infected handlers and/or poor hygiene practices. As they are often eaten raw, the presence of pathogens in leafy herbs creates a potential risk for foodborne illness.

Considering the above factors and their relevance to Canadians, leafy herbs have been selected as one of the priority commodity groups of fresh fruits and vegetables for enhanced surveillance. Over the course of five years (2009/10-2013/14) of targeted surveys on leafy herbs, approximately 7,000 leafy herb samples were collected from Canadian retail locations and tested for the presence of pathogens of concern.

The main objectives of the 2012/13 and 2013/14 targeted surveys were to generate baseline surveillance data on bacterial pathogens of concern *Salmonella*, *Shigella*, *Escherichia coli* (*E. coli*) O157:H7/NM (non-motile), and *Campylobacter*, as well as generic *E. coli* (an indicator of fecal contamination) for leafy herbs available in the Canadian market. In total, 2,472 fresh leafy herb samples were collected and analysed. The majority (99.5%) of the samples were assessed as satisfactory. Eight samples (0.3%) were unsatisfactory; one sample was contaminated with *Salmonella* and seven other samples had high levels of generic *E. coli* (> 1,000 Most Probable Number (MPN)/g). Subsequent food safety investigations resulted in no product recalls. It is important to note that there were no reported illnesses associated with consumption of any of the *Salmonella* contaminated

product during this survey. In addition, five samples (0.2%) had elevated, yet marginally acceptable, levels of generic *E. coli* (100 – 1,000 MPN/g). These samples were assessed as investigative and further evaluation resulted in no immediate follow-up activities. These findings suggest that the majority of leafy herbs 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 of 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 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 7,000 leafy herb samples over five years (2009/10 to 2013/14) and was designed to gather baseline information on the occurrence of bacterial pathogens of concern in leafy herbs 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 Agriculture 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 labelled.

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, bacterial pathogens and/or high levels of generic *E. coli* detected in any samples tested under these surveys would 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 Herbs

2.1 Rationale

Leafy herbs have been reported to be responsible for numerous outbreaks of foodborne illness worldwide. From 1997 to March 2014, 23 foodborne disease outbreaks associated with leafy herbs contaminated with bacterial pathogens were documented worldwide (information based on data compiled by the Public Health Agency of Canada (PHAC), Appendix B). Of these outbreaks, pathogenic *Escherichia coli* (*E. coli*), *Shigella* and *Salmonella* were identified and accounted for approximately 95% of the outbreaks (Appendix C). Three outbreaks occurred in Canada that were linked to leafy herbs contaminated with *Shigella sonnei* (Appendix B).

Like leafy vegetables, both organic and conventional leafy herbs are available on the Canadian market place. Leafy herbs can become contaminated with various foodborne pathogens in the field by domestic and wild animals, improperly composted manure, and contaminated irrigation water during primary production. Leafy herbs can also become contaminated with pathogens during harvest, post-harvest handling, processing, storage and distribution by poor hygiene practices and/or infected handlers. As leafy herbs are often consumed raw, contaminated leafy herbs can cause foodborne illnesses.

Contaminated leafy herbs can introduce pathogens from an herb producing country to herb-consuming countries resulting in outbreaks of foodborne illness. Recent outbreaks of foodborne illness that occurred in the United Kingdom[7,8], Denmark [9], and Norway [10] were associated with imported leafy herbs harbouring bacterial pathogens (e.g., *Salmonella*, pathogenic *E. coli*, and *Shigella*).

Leafy herbs, along with leafy vegetables, were identified as a level one (highest) priority of concern among fresh fruits and vegetables in terms of microbiological hazards during a 2007 joint FAO/WHO Expert Meeting [11]. 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 in a wide range of geographic locations).

Based on the above information and the Food Safety Science Committee's recommendations [12], fresh leafy herbs were selected for targeted surveillance between the 2009/2010 and 2013/14 fiscal years. The overall objective was to gather baseline information on the occurrence of various pathogens of concern in leafy herbs available to Canadians at retail. These targeted surveys (2012/13 & 2013/14) were part of the information collection with a focus on investigating the presence and distribution of bacterial pathogens *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter*, 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 herbs.

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 [13]. Most outbreaks associated with these bacterial pathogens are linked to the consumption of contaminated food of animal origin (e.g., chicken and beef, respectively). However, in the last decade, fresh fruits and vegetables have emerged as significant sources of illnesses related to these bacteria [14]. Fruits and vegetables can become contaminated with these bacterial pathogens in the field by improperly composted manure, contaminated water, wildlife feces, and/or poor hygiene practices of the farm workers [15].

Humans are the only host of the bacterial pathogen *Shigella*. Food contaminated by infected food handlers 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 [13].

Similar to *Salmonella* and *E. coli* O157:H7, bacterial pathogen *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. [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* [13].

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 [18]. The presence of generic *E. coli* in foods can also indicate potential contamination with pathogenic enteric microorganisms, such as *Salmonella* or *E. coli* O157:H7, 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 is an indication that contamination has occurred at some point between production and the time of sale.

2.3 Sample Collection

Leafy herb samples included pre-trimmed bunches, or pre-packaged, non-cut fresh leafy herbs. Dried herbs were excluded from this survey.

All samples were collected from national chain and local/regional grocery stores, other conventional retail, natural food stores and farmers' market 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 collected during June - November. Imported samples were collected year round. Samples that were labelled 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 minimum weight of 150 g. This sampling approach has been used for many retail food surveys [19, 20, 21] and by other federal partners such as the Public Health Agency of Canada (PHAC) under the retail component of their FoodNet surveys [22].

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 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 herbs.

The assessment criteria presented below (Table 1 and Table 2) are based on principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* [24] and associated methods published in Health Canada's *Compendium of Analytical Methods* [23].

Table 1 Assessment Guidelines for Bacterial Pathogens in Leafy Herbs

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

* *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, 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 Herbs

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**		
	Satisfactory	**Investigative**	**Unsatisfactory**
Generic *E. coli* (MFHPB-19 or 27)**	≤ 100	$100 < x \leq 1000$	> 1000

* *Compendium of Analytical Methods* [23].

** Unit for MFHPB-19 method: MPN/g, for MFHPB-27 method: CFU/g. MFHPB-19 method was used for the majority of the samples in these surveys.

Samples assessed as investigative in this survey required some form of follow-up activity. For example, further sampling may be done to verify the levels of generic *E. coli* in the products in question. Unsatisfactory sample assessments were subject to follow-up actions, such as directed follow-up sampling, inspection of establishment, health risk assessment, and/or product action (e.g., product recall).

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 these surveys 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. Likewise, differences between production practices (organic and conventional samples) were not analyzed in this report.

3 Results

3.1 Sample Distribution by Country of Origin

As per the survey design, imported and domestic, conventional and organically grown fresh leafy herbs were collected. Approximately one third of the herb samples were domestically produced and two thirds of the herb samples were imported (Table 3). The imported herb samples were mainly from the U.S. and Mexico, as well as nine other countries. The country of origin could not be identified for 33 samples (1.3%).

Table 3 Sample Distribution by Country of Origin

Country of Origin	Conventional	Organic	Total	
	Number of Samples	Number of Samples	Number of Samples	Percentage of Total
Canada	519	357	876	35.4
Subtotal – Domestic	519	357	876	*35.4*
Colombia	43	32	75	3.0
Costa Rica	3	0	3	0.1
Dominican Republic	38	3	41	1.7
Ecuador	1	0	1	0.04
Israel	34	5	39	1.6
Mexico	134	100	234	9.5
Morocco	2	0	2	0.1
Tanzania	1	0	1	0.04
Thailand	1	0	1	0.04
USA	478	683	1161	47.0
Vietnam	5	0	5	0.2
Subtotal - Imported	*740*	*823*	*1563*	*63.2*
Unidentified	7	26	33	1.3
Total	**1266**	**1206**	**2472**	**100**

3.2 Sample Distribution by Product Type

More than 15 different types of fresh herbs were collected from the Canadian retail market. Five types of herbs, parsley, cilantro (coriander), dill, basil and mint, accounted for majority (85.8%) of the herb samples collected in this survey (Table 4).

Table 4 Types of Fresh Leafy Herb Samples

Type of Herb	Conventional	Organic	Total	
	Number of Samples	Number of Samples	Number of Samples	Percentage of Total (%)
Basil	70	71	141	5.7
Chives	42	22	64	2.6
Cilantro	231	234	465	18.8
Dill	141	83	224	9.1
Marjoram	5	2	7	0.3
Mint	77	52	129	5.2
Oregano	37	14	51	2.1
Parsley	540	621	1161	47.0
Rosemary	26	32	58	2.3
Sage	32	17	49	2.0
Savoury	9	4	13	0.5
Sorrel	3	0	3	0.1
Tarragon	9	11	20	0.8
Thyme	21	26	47	1.9
Wheatgrass	0	11	11	0.4
Others *	23	6	29	1.2
Total	**1266**	**1206**	**2472**	**100.0**

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

3.3 Assessment Results

A total of 2,472 herb samples were analysed for pathogenic bacteria *E. coli* O157:H7/NM, *Salmonella*, *Shigella*, and *Campylobacter*, as well as generic *E. coli*, an indicator of fecal contamination.

E. coli O157:H7, *E. coli* O157:NM, *Shigella* and *Campylobacter* were not detected in any of the herb samples tested. *Salmonella* and generic *E. coli* (> 100 MPN/g) were not found in the majority of the samples (99.5%) (Table 5).

Table 5 Summary of Assessment Results of Fresh Leafy Herb Samples

Product Origin	Production Practice	Number of Samples	Assessment		
			Unsatisfactory	Investigative	Satisfactory
			Number of Samples (Percentage)	Number of Samples (Percentage)	Number of Samples (Percentage)
Imported	Conventional	740	2	2	736
	Organic	823	2	0	821
	Subtotal	***1563***	***4***	***2***	***1557***
Domestic	Conventional	519	2	2	515
	Organic	357	1	1	355
	Subtotal	***876***	***3***	***3***	***870***
Unknown	Conventional	7	0	0	7
	Organic	26	1	0	25
	Subtotal	***33***	***1***	***0***	***32***
Total		**2472 (100%)**	**8 (0.3%)**	**5 (0.2%)**	**2459 (99.5%)**

Eight samples (0.3%) were found to be unsatisfactory (Table 6). One sample was unsatisfactory due to the presence of *Salmonella* and the other seven samples had high levels of generic *E. coli*. *Salmonella* Sandiego was identified from the isolate of the *Salmonella* positive sample. The *Salmonella* contaminated sample was from Mexico. The other unsatisfactory samples originated from different countries as indicated in Table 6.

As a result of these findings, the CFIA conducted food safety investigations and appropriate follow-up activities for the unsatisfactory samples. No product recalls resulted from the unsatisfactory samples and subsequent food safety investigations. It is important to note that there were no reported illnesses associated with consumption of any of the *Salmonella* contaminated product during this survey.

Table 6 Summary of Unsatisfactory Samples

Product Type/Production Practice/ Country of Origin	**Reason for Unsatisfactory Assessment**
Basil/Organic/Mexico	*Salmonella* Sandiego
Dill/Conventional/Canada	Generic E. coli: >1600 MPN/g
Parsley/Conventional/Canada	Generic *E. coli*: >1600 MPN/g
Tarragon/Organic/Canada	Generic *E. coli*: >1600 MPN/g
Basil/Conventional/USA	Generic E. coli: >1600 MPN/g
Mint/Organic/Colombia	Generic E. coli: >1600 MPN/g
Dill/Conventional/USA	Generic E. coli: >1600 MPN/g
Mint/Organic/Unknown	Generic E. coli: >1600 MPN/g

Elevated levels of generic *E. coli* (> 100 and ≤ 1,000 MPN/g) were found in a total of five samples (0.2%) (Table 7). These samples were assessed as investigative, as the *E. coli* counts were elevated but below the unsatisfactory threshold. Further evaluation of these samples resulted in no immediate follow-up actions.

Table 7 Summary of Investigative Samples

Product Type /Production Practice /Country of Origin	**Generic *E. coli* Counts (MPN/g)**
Sage/Conventional/Mexico	540
Dill/Conventional/Canada	350
Mint/Conventional/Mexico	220
Parsley/Conventional/Canada	130
Parsley/Organic/Canada	130

4 Discussion and Conclusion

In these surveys (2012/13 & 2013/14), *E. coli* O157:H7/NM, *Shigella*, and *Campylobacter* were not detected in any of the 2472 leafy herb samples tested. The majority (99.5%) of the samples were assessed as satisfactory. However, *Salmonella* was detected in one sample (0.04%), high levels of generic *E. coli* (> 1,000 MPN/g) were found in seven samples (0.28%) and elevated levels of generic *E. coli* (100 - 1,000 MPN/g) were found in five samples (0.20%).

As a result of the unsatisfactory findings, the CFIA conducted appropriate food safety investigations including directed sampling, inspection of facilities or review of importation procedures, and health risk assessment (conducted by Health Canada). No product recalls resulted from the subsequent food safety investigations. It is important to note that there were no reported illnesses associated with the *Salmonella* contaminated product during this survey. After further evaluation of the investigative results, no further actions were deemed necessary.

The overall findings of this survey suggest that fresh leafy herbs in the Canadian market are generally produced and handled under acceptable GAPs/GMPs. However, contamination of leafy herbs with *Salmonella* can occur at a very low rate, and can represent a food safety risk. The presence of elevated or high levels of generic *E. coli* in leafy herbs can also occur. Although generic *E. coli* are not disease causing agents, their presence is used by the CFIA as an indicator to assess general sanitation and hygiene practices throughout the production chain.

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 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 Acknowledgement

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Appendix A: List of Acronyms

CDC: Centres 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

FSSC: Food Safety Science Committee

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

***Salmonella* spp.**: *Salmonella* 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 Fresh Leafy Herbs Contaminated with Bacterial Pathogens (1997 – March 2014)*

Case number	Year	Product	Micro-organism	Country	Number of Cases	Source
1	1998	Parsley	Multiple Organisms	Multiple Countries	1126	J Food Protection 2003;66(4):535-541
2	1998	Parsley	*Shigella boydii*	Massachusetts, USA	6	Journal of Food Protection 2003, 66(4):535-541 & JFP 68 (3):521-527
3	1998	Parsley	*Shigella boydii*	Florida, USA	37	Journal of Food Protection 2003, 66(4):535-541 & JFP 68 (3):521-527
4	1998	Parsley	*Shigella sonnei*	Alberta, Canada	4	Journal of Food Protection 2003, 66(4):535-541
5	1998	Parsley	*Shigella sonnei*	Ontario, Canada	35	Morbidity and Mortality Weekly Report (MMWR) 1998, 48(14) :285-9
6	1998	Parsley	*Shigella sonnei*	British Columbia, Canada	13	Canada Communicable Disease Report 1999, Vol 25
7	1998	Parsley	*Shigella sonnei*	California, USA	9	J Food Protection 2003; 66(4):535-541
8	1998	Parsley	*E. coli* O6:H16	Minnesota, USA	42	Emerging Infectious Diseases 2004, 10(3) & Journal of Food Protection 2003; 66(4):535-541
9	1998	Parsley	Enterotoxigenic *E. coli*	Minnesota, USA	35	J Food Protection 2003; 66(4):535-541
10	1999	Cilantro	*Salmonella* Thompson	California, USA	35	CDC
11	1999	Basil	*Shigella sonnei*	Multiple States, USA	10	CDC
12	2000	Basil	*E. coli* O169:H41	Washington, USA	100	Emerging Infectious Diseases Vol. 10; No. 3, 2004

Case number	Year	Product	Micro-organism	Country	Number of Cases	Source
13	2001	Cilantro	*Salmonella* Newport	California, USA	8	CDC
14	2002	Cilantro	*Salmonella* Newport	Colorado, USA	13	CDC
15	2005	Parsley	*E. coli* O157:H7	Oregon, USA	18	ProMed Oct. 25, 2005 & FSNet Oct. 31 2005
16	2005	Parsley	*E. coli* O157:H7	Washington, USA	4	CDC 2005
17	2005	Parsley	*E. coli* O157:H7	Washington, USA	2	CDC 2005
18	2006	Basil	Enterohemorrhagic *E. coli*	Denmark	250	European Food Safety Authority
19	2007	Basil	*Salmonella* Senftenberg	United Kingdom	32	Foodborne Pathogens and Disease, Vol 5, No 5
20	2007	Basil	*Salmonella* Senftenberg	Multiple states, USA	11	CDC 2007
21	2009	Parsley	*E. coli* O157	South Australia	31	OzFoodNet quarterly report, 2009: Oct-Dec
22	2011	Basil	*Shigella sonnei*	Norway	46	EID, 18:9 2012
23	2013	Curry Leaves	*Salmonella* multiple serotypes	Newcastle, UK	413	Public Health England (ILOG 8168)

*Information of the outbreaks was compiled 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 Herbs Contaminated with Bacterial Pathogens (1997 – March 2014)

Bacterial Pathogen	Outbreaks	
	Number of Outbreaks	Percentage of Outbreaks
Pathogenic *E. coli*	8	34.8
***Salmonella* spp.**	6	26.1
***Shigella* spp.**	8	34.8
Multiple pathogenic bacteria	1	4.3
Total	**23**	**100**

* Summarized according to Appendix B

Appendix D: Analytical Methods Used for Microbial Analysis

Bacterial 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
***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
***Campylobacter* spp.**	(MFLP-46) (March 2002, modified***)	Isolation of Thermophilic *Campylobacter* from Food
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and *E. coli* in Foods
	MFHPB-27 (September 1997)	Enumeration of *Escherichia coli* in Foods by the Direct Plating (DP) Method

**Compendium of Analytical Methods*[25].

** 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.

*** 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.