Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2009-2010 Targeted Surveys

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

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 herbs have been reported to be responsible for numerous global outbreaks of foodborne illness. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has ranked leafy herbs, along with leafy vegetables, as the highest priority in fresh fruits and vegetables in terms of microbiological hazards. After harvest, leafy herbs are subject only to minimal processing (e.g., cutting, trimming, sanitizing, washing, packaging) and are often consumed raw. As such, pathogens introduced during any step of production may not only survive but also multiply. The bacterial pathogens *Salmonella*, Verotoxin-producing *Escherichia coli (E. coli)* (VTEC), and *Shigella* were identified to be related to many outbreaks of foodborne illness associated with leafy herbs.

Considering these 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 under the FSAP.

During four annual microbiological targeted surveys (2009/10 – 2012/13), over 5000 leafy herb samples will be collected and tested for the presence of pathogens of concern in leafy herbs available to Canadians at retail. This targeted survey (2009/10) focused on bacterial pathogens of concern and generic *E. coli* (as indicator bacteria of fecal contamination). The main objectives of this survey were to generate baseline surveillance data on:

- the presence and distribution of bacterial pathogens of concern: *E. coli* O157:H7/NM, *Salmonella* species (spp.), and *Shigella* spp.; and
- the presence, distribution and levels of generic *E. coli*

in fresh leafy herbs available in the Canadian retail market.

In this survey, a total of 1,224 retail samples of leafy herbs were collected and analysed, including 816 imported and 408 domestically produced samples. Pathogenic bacteria and generic *E. coli* were not detected in the majority of samples (98.4%). A total of nine samples (0.7%) were found to be unsatisfactory. One sample (0.08%) was unsatisfactory due to the presence of *Salmonella* and the other eight samples (0.7%) were unsatisfactory due to high levels of generic *E. coli* (>1000 colony forming units (CFU)/g). *E. coli* O157:H7, *E. coli* O157:NM (non-motile), and *Shigella* spp. were not detected in any of the herb samples in this survey. In addition, elevated levels of generic *E. coli* were

found in 10 samples (0.8%). These samples were assessed as investigative and requiring further evaluation given that *E. coli* counts were elevated but below the unsatisfactory threshold of 1000 CFU/g. Evaluation of these samples did not result in any follow-up activities. To assist in the food safety investigations, the serotypes and pulsed field gel electrophoresis (PFGE) pattern (i.e., DNA typing) were identified from the isolates submitted from the one *Salmonella* positive sample.

Of the nine unsatisfactory samples, eight were imported (8/816, 1.0%) and one was domestically produced (1/408, 0.2%). The unsatisfactory imported herb samples originated from Mexico, the United States, and the Dominican Republic. All unsatisfactory samples were subject to food safety investigations and appropriate follow-up activities were conducted by the CFIA. One product recall resulted from the referral of the unsatisfactory samples and subsequent investigations. It is important to note that there were no reported illnesses associated with the consumption of the *Salmonella*-contaminated product in this survey.

Results of the 2009-10 survey indicate that the vast majority (98.4%) of the leafy herb samples were not contaminated with bacterial pathogens or with generic *E. coli*, an indicator used by the Agency to assess general sanitation and hygiene practices throughout the production chain. The study revealed that a low percentage (1.6%) of the leafy herb samples was contaminated by microorganisms that could represent a potential source of foodborne illness; however, no reported illnesses were associated with consumption of any of the product type in this survey during the time of the study. Contamination in these few samples could be the result of inadequate practices during production, packaging, or storage. Results of the three remaining microbiological targeted surveys on leafy herbs will be released annually upon completion.

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. The CFIA as well as other jurisdictions such as provincial and municipal regulators do monitor the controls throughout the food production chains. 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 enhance the food safety regulatory system. The FCSAP initiative unites multiple partners in ensuring safe food for Canadians.

The Canadian Food Inspection Agency's (CFIA's) Food Safety Action Plan (FSAP) (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 that these risks occur, improve import and domestic food controls, and identify food importers and manufacturers.

Within the FSAP, there are twelve 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

Microbiological targeted surveys are used to gather information regarding the potential occurrence of microbiological hazards in defined food commodities. The surveys are designed to focus on priority and/or emerging food hazard issues and to address areas not covered by regular CFIA monitoring activities.

The microbiological targeted surveys aim to establish baseline data on priority microbiological hazards in targeted commodities, primarily fresh fruits and vegetables and imported food ingredients. Based on the priority of microbiological risk associated with particular commodity groups and to account for seasonal variation and other factors, a statistically significant number of leafy herb samples will be collected over the four years of planned FSAP targeted surveys. This work differs from regular CFIA microbiological monitoring activities, which tests samples of a broad range of commodities for multiple hazards.

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 microbiological targeted survey (2009/10) represents the first phase of collection of over 5000 leafy herb samples over four years (2009/10 – 2012/13). This survey tested for the presence of bacterial pathogens of concern and the presence and levels of generic *E. coli* (as indicator bacteria of fecal contamination) in imported and domestically produced fresh leafy herbs available in the Canadian market.

1.3 Codes of Practice, Acts and Regulations

Food safety standards are developed under the joint FAO/WHO Food Standards Programme. Producers of fresh fruits and vegetables (including fresh leafy herbs) are encouraged to follow the international codes of practice developed by the Codex Alimentarius Committee, which provide guidance for the safe production of the food. Of relevance for this survey are the *Code of Hygienic Practices for Fresh Fruits and Vegetables* (CAC/RCP 53-2003) and *Recommended International Codes of Practice-General Principles of Food Hygiene* (CAC/RCP 1-1969) (4,5). These codes address GAP and 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. They outline basic requirements pertaining to environmental hygiene, hygienic production (water, manure, soil biological control, packing, facility and personal hygiene), handling, storage, transportation, maintenance and sanitation.

Fresh fruits and vegetables (including fresh leafy herbs) available in the Canadian market must comply with the *Food and Drugs Act* (FDA) and the *Food and Drug Regulations* (FDR), 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 sold in Canada must also comply with safety requirements of the *Fresh Fruit and Vegetable Regulations* under the *Canada Agricultural Products Act.* These regulations are intended to ensure that fresh fruits and vegetables sold to consumers are safe, wholesome, properly graded, packaged and labelled. Both the *Fresh Fruit and Vegetable Regulations* and the food-related portions of the FDA and FDR are enforced by the CFIA.

FSAP targeted surveys are conducted for surveillance and not for regulatory purposes. However, bacterial pathogens and/or excessive levels of generic *E. coli* detected in any samples tested under this survey 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 Description of Hazards and Survey Details

2.1 Microbiological Hazards in Fresh Leafy Herbs

As leafy herbs are commonly consumed raw and often used as a food flavouring ingredient, they are considered ready-to-eat foods, even through there could be washing or cooking involved in the preparation. Leafy herbs, like other leafy vegetables, can become contaminated with various foodborne pathogens. Due to historical outbreaks, potential for contamination during pre-harvest, post-harvest and distribution, along with other evidence, leafy herbs and leafy green vegetables were identified as a level one (highest) priority in fresh fruits and vegetables in terms of microbiological hazards during a 2007 joint FAO/WHO Expert Meeting (6).

2.1.1 Foodborne Disease Outbreaks Associated with Fresh Leafy Herbs Contaminated with Bacterial Pathogens

From 1997 to 2009, 20 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), Table 1, Appendix B). Of these outbreaks, Verotoxin-producing *E. coli* (VTEC), *Salmonella* spp., and *Shigella* spp. were almost evenly identified (Table 1). The leafy herbs implicated in the outbreaks were basil (25%), parsley (65%) and coriander (cilantro) (10%). Three outbreaks in Canada have been linked to leafy herbs contaminated with *Shigella sonnei*.

Table 1. Global Foodborne Disease Outbreaks Associated with Fresh Leafy Herbs Contaminated with Bacterial Pathogens (1997-2009)*

Bacterial Pathogen	Outbreaks	
	Number of Outbreaks	Percentage of Outbreaks
Pathogenic *E. coli*	6	30
***Salmonella* spp.**	5	25
***Shigella* spp.**	7	35
Multiple pathogenic bacteria	2	10
Total	**20**	**100**

* Summarized according to Appendix B

Recent foodborne disease outbreaks that occurred in the United Kingdom (UK), the United States (U.S.) and Denmark were associated with the international trade of leafy herbs harbouring bacterial pathogens (7-9). The contaminated leafy herbs introduced pathogenic *E. coli* and *Salmonella* spp. from the herb-producing country to the herb-consuming countries (UK, U.S. and Denmark) resulting in foodborne disease outbreaks (7-9).

2.1.2 Bacterial Pathogens in Fresh Leafy Herbs

Publicly available information was also used to assess what bacterial pathogens were seen in leafy herb surveys carried out in other jurisdictions. While these results may not be directly transferable to Canada, they are considered to be representative of similar production practices and may be viewed as good indicators of what might be found.

Available survey data on bacterial pathogens in leafy herbs from the UK and the US also suggest microbiological risk associated with leafy herbs, especially imported leafy herbs. A microbiological survey of leafy herbs in the UK retail market was performed in 2007 in response to an outbreak (8). Results of the survey indicated that 1.6% (60/3760) of leafy herbs were unsatisfactory due to the presence of *Salmonella* and/or generic *E. coli* (> 1000 CFU/g), and *Salmonella* spp. were detected in 0.5% (18/3760) of samples (other bacterial pathogens were not tested in this study) (8). Similarly, U.S. Food and Drug Administration (US FDA) surveys testing bacterial pathogens in non-retail fresh produce (including leafy herbs of cilantro and parsley) from 1999 to 2000 found unsatisfactory rates of 1.1% (2/175) and 5.3% (23/438) in U.S. domestic and imported leafy herbs, respectively (10,11). *Salmonella* spp. were found in 0.6 % (1/175) of U.S. domestic and 5.0% (22/438) of U.S. imported leafy herbs (10,11). *Shigella* spp. were detected in one sample each of U.S. domestic (0.6%) and imported (0.2%) leafy herbs. *E. coli* O157:H7 was not detected in any of the samples in these US FDA surveys (10,11).

The bacterial pathogens of concern *E. coli* O157:H7/NM, *Salmonella* spp., and *Shigella* spp., as well as generic *E. coli* as indicator bacteria of fecal contamination were targeted in this survey.

2.2 Bacterial Pathogens of Concern and Generic *E. coli*

2.2.1 Pathogenic *E. coli*

A few strains of *E. coli* are capable of causing human disease. Based on the disease syndromes and characteristics, there are currently five recognized classes of pathogenic *E. coli* that cause gastroenteritis in humans: enteroaggregative, enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic *E. coli* (12,13). The enterohemorrhagic *E. coli* are Verotoxin-producing *E. coli* (VTEC) that can produce

Shiga-like toxin and cause severe diarrhea. This class of *E. coli* includes the predominant disease-causing *E. coli* O157:H7/NM (14) and other emerging disease-causing non-O157 *E. coli*.

Infections caused by VTEC are notifiable infectious diseases that are reported to the PHAC in Canada and Centers for Disease Control (CDC) in the U.S. In Canada, 1103 cases of VTEC infections were reported in 2004 and the majority (94%) of the VTEC infections were caused by *E. coli* O157 serotype (15). Similarly, in the U.S., a total of 2348 laboratory-confirmed cases of *E. coli* O157 infections (91%) and 224 non-O157 VTEC infections (9%) were reported in 2005 (16). It is notable that non-O157 VTEC infection cases have increased each year since 2001 when identification of non-O157 VTEC was requested by CDC in the U.S. (16).

E. coli O157 bacteria are found naturally in the intestines of ruminant animals, such as cattle, sheep and deer, as well as other animals, such as rabbits and pigs. Most outbreaks associated with *E. coli* O157 are linked to consumption of contaminated food or water. While ground beef is still the most common food source of *E. coli* O157 associated foodborne illnesses, fresh fruits and vegetables have also emerged as significant sources of *E. coli* O157 related illnesses in the last decade. Fruits and vegetables can be contaminated with *E. coli* O157 in the field by improperly composted manure, contaminated water, wildlife feces, or poor hygienic practices of the farm workers (17).

2.2.2 *Salmonella* spp.

There are over 2,500 serotypes of *Salmonella* (spp.), many of which are capable of causing human disease, known as salmonellosis.

Salmonellosis is one of the most common foodborne illnesses worldwide. The incidence of human salmonellosis varies depending on geographic, demographic, socioeconomic and environmental factors. There were approximately 6,000 cases of *Salmonella* infection reported in Canada annually from 2000 to 2004, according to available information from the PHAC (15). It is believed that the actual number of infections is much higher due to under reporting, meaning that only a fraction of the true number of cases are reported (15). In the U.S., approximately 30,000 to 40,000 laboratory–confirmed cases of salmonellosis were reported to the CDC each year between 2000 and 2009 (18). However, it is estimated that 1.4 million cases of *Salmonella* infection occur annually, and that the annual cost associated with these illnesses represent approximately US $2.7 billion (18, 19).

Salmonella normally live in the intestines of animals such as poultry, swine, wild birds, domestic pets and reptiles. Therefore, *Salmonella* contamination often occurs in food of

animal origin (e.g., meat, poultry, eggs and milk). *Salmonella* spp. excreted in animal feces remain viable in the field for a relatively long period of time. Produce grown in fields that contain improperly composted manure or contaminated water can therefore become contaminated. Foodborne illnesses of salmonellosis have been increasingly reported to be associated with the consumption of contaminated fruits and vegetables.

2.2.3 *Shigella* spp.

Shigella species belong to a large family of Gram-negative bacteria, as do the *Salmonella* spp. and *E. coli*. There are four groups or species of *Shigella* known to cause shigellosis: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. *Shigella dysenteria* is considered the most virulent group of *Shigella*, as this group of *Shigella* can produce Shiga toxin that destroys cellular function and causes severe bloody diarrhea. *Shigella sonnei* and *S. flexneri* are the most common causes of shigellosis in Canada and the U.S.

The annual number of shigellosis illnesses was estimated to be about 120 million worldwide, with the majority of the infections occuring in developing countries according to information reported by the WHO in 2009 (20). In Canada, 1156 cases of *Shigella* infection were reported in 2000 and 720 cases were reported in 2004 (15). The reported cases declined overall between 2000 and 2004, with the exception of a spike in 2002 (1355 cases/year). The elevated cases in 2002 related to a foodborne outbreak of *Shigella sonnei* in pasta salad in Ontario (15). In 2006 in the U.S., a total of 10,336 laboratory-confirmed cases of *Shigellosis* were reported, translating to an average national incidence of 3.5 cases per 100,000 people (21). Data available since the analysis of this survey was completed indicate that shigellosis remains a common infectious disease worldwide.

Since humans are the only host of *Shigella* spp., shigellosis (also called bacillary dysentery) is principally a human disease and rarely occurs in animals. *Shigella* infection can spread from person to person under poor hygienic conditions including inadequate hand-washing habits. 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.

2.2.4 Generic *E. coli* as Indicator Bacteria of Fecal Contamination

Typically, *E. coli* bacteria that inhabit the large intestines of humans and animals are harmless. Due to their regular presence in stools of humans and animals, the occurrence of *E. coli* in foods indicates direct or indirect contamination with fecal matter. The presence of generic *E. coli* in foods can also indicate potential contamination with pathogenic enteric 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 the increased risk of contamination with pathogenic micro-organisms but does not conclusively indicate that these pathogenic organisms are present. High levels of generic *E. coli* in fresh produce sold at retail as an indication that contamination may have occurred at some point between production and the time of sale.

2.3 Rationale

Considering a range of factors, including historical outbreaks of foodborne illness and their relevance to Canadians, as well as microbiological risk, leafy herbs have been selected as one of the priority commodity groups of fresh produce for targeted surveillance under FSAP between 2009/10 to 2012/13. The overall objective of the targeted surveys is to gather baseline information on the occurrence of pathogens of concern (pathogenic bacteria, viruses and parasites) and indicator bacteria (generic *E. coli*) in leafy herbs available to Canadians at retail. The 2009-10 targeted survey is the first phase of data collection with a focus on investigating the presence of bacterial pathogens (*E. coli* O157:H7/NM, *Salmonella*, and *Shigella*) and the presence and levels of generic *E. coli* (as indicator bacteria of fecal contamination) in imported and domestically produced leafy herbs.

2.4 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 and natural food stores located across Canada. The number of samples collected in various geographic regions in Canada 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.

In this survey, a sample represented a single sample unit (e.g. an individual consumer size package) with a total weight of no less than 200 g. Collected samples were required to be shipped under conditions that limited the growth of micro-organisms during transit.

2.5 Sample Distribution

A total of 1224 samples were collected, including 816 (66.7%) imported and 408 (33.3%) domestically produced leafy herbs.

The imported herb samples originated from six countries (Figure 1). Approximately half of the herb samples were imported from the U.S., and the remaining half of the samples were imported from five other countries: Colombia, Costa Rica, Dominican Republic, Israel and Mexico.

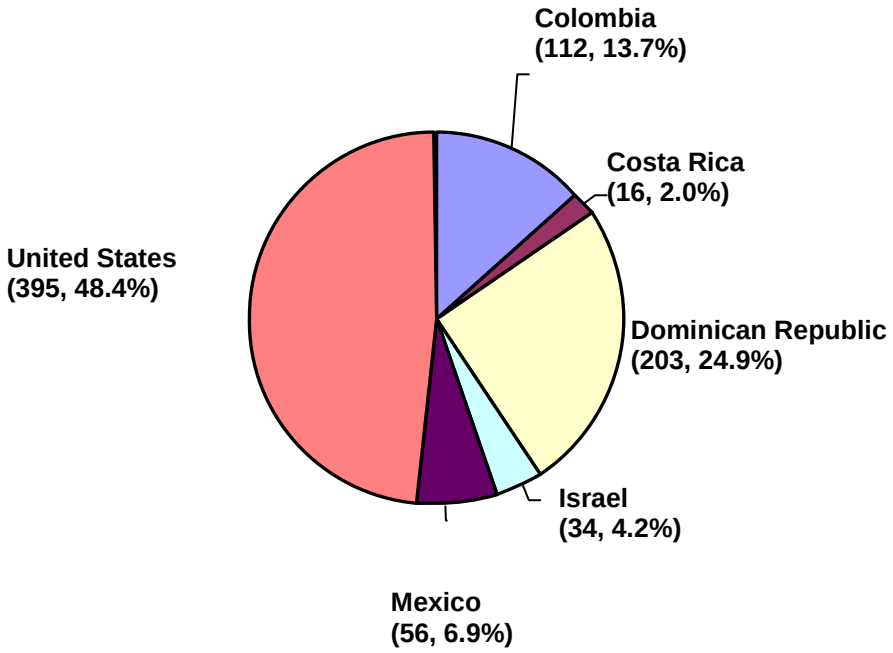

Figure 1. Distribution of Imported Herb Samples by Country of Origin (number of samples, percentage of samples)

More than 14 different types of fresh herbs were collected from the Canadian retail market (Table 2).

Table 2. Distribution of Fresh Leafy Herb Samples by Product Type

Product Type	Imported		Domestic		Total	
	Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples
Basil	134	16.4	79	19.4	213	17.4
Chives	36	4.4	23	5.6	59	4.8
Cilantro	99	12.1	62	15.2	161	13.2
Dill	9	1.1	2	0.5	11	0.9
Marjoram	42	5.2	2	0.5	44	3.6
Mint	3	0.4	12	2.9	15	1.2
Oregano	46	5.6	17	4.2	63	5.2
Parsley	185	22.7	130	31.9	315	25.7
Rosemary	67	8.2	15	3.7	82	6.7
Sage	59	7.2	16	3.9	75	6.1
Savoury	29	3.6	13	3.2	42	3.4
Sorrel	32	3.9	6	1.5	38	3.1
Tarragon	55	6.7	12	2.9	67	5.5
Thyme	13	1.6	13	3.2	26	2.1
Other*	7	0.9	6	1.5	13	1.1
Total	**816**	**100**	**408**	**100**	**1224**	**100**

* Other refers to herb types with small numbers of samples.

2.6 Method Details

All samples were analysed using the analytical methods published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods (22) (Appendix C). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of fresh fruits and vegetables.

For the detection of *E. coli* O157:H7/NM, *Salmonella*, and *Shigella*, a two-step procedure was employed. Samples were first screened by polymerase chain reaction (PCR)-based methods. Any presumptive positive results required confirmation by isolation, purification and identification procedures.

If any pathogens were detected, these isolates were further characterised by pulsed field gel electrophoresis (PFGE) (i.e., DNA typing) at the CFIA Ottawa Laboratory (Fallowfield). Serotyping for *Salmonella* spp. was performed at the *Salmonella* Typing Laboratory, Laboratory for Foodborne Zoonoses, PHAC in Guelph, Ontario.

The count of generic *E. coli* was accomplished by the most probable number (MPN) or direct plating procedure.

2.7 Assessment Guidelines

The assessment criteria for the targeted surveys are based on the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* (23) and associated methods published in Health Canada's *Compendium of Analytical Methods* (22). The presence or absence of *E. coli* O157:H7/NM, *Salmonella*, or *Shigella* was determined from a 25 g sample unit drawn from a sample submitted for analysis. A positive result (presence in 25 g) was assessed as unsatisfactory, while a negative result (absence in 25 g) was assessed as satisfactory (Table 3).

Table 3. Assessment Guidelines for Pathogenic Bacteria in Fresh Leafy Herbs

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
***E. coli* O157:H7/NM** (MFLP-30, Supplement 1 & 2, and MFLP-80)	Absent in 25 g	Present in 25 g
Salmonella* spp.* (MFLP-29 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

* *Compendium of Analytical Methods* (22).

** No criteria have been established by Health Canada at this time for either *Salmonella* or *Shigella* in fresh fruits and vegetables. However, their presence in these foods is considered to be a violation of FDA Section 4(1)a and is therefore assessed by the CFIA as unsatisfactory.

A satisfactory assessment for the generic *E. coli* in this survey indicated that the organisms were either not detected in the count, or found at very low levels (i.e. $\leq$ 100 CFU/g). *E. coli* counts in the range of 100 - 1000 CFU/g were assessed as investigative and required some form of follow-up activity. For example, further sampling may have been done to verify the levels of generic *E. coli* in the samples in

question. Results indicating *E. coli* levels above 1000 CFU/g were assessed as unsatisfactory (Table 4).

Table 4. Assessment Guidelines for Generic *E. coli* in Fresh Leafy Herbs

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

* *Compendium of Analytical Methods* (22).

** Concentration unit depends on method used. For MFHPB-19 method: MPN/g (Most Probable Number/gram), for MFHPB-27 method: CFU/g (Colony Forming Unit/gram).

An unsatisfactory sample assessment was subject to follow-up actions, such as food safety investigation, directed follow-up sampling, inspection of establishment, health risk assessment, and/or product action (e.g. product recall).

2.8 Survey Limitations

All samples in this survey were collected at retail to enable collection of information on the presence and levels of microbiological hazards in leafy herbs available to Canadian consumers. However, sampling at retail also imposes certain limitations with respect to the traceability of products in the case of positive results. For example, it may be difficult to identify the source of contamination since the samples were collected from bulk or from units pre-packaged at packaging facilities or at the retail level.

In addition, to properly assess the compliance of a lot against microbiological standards, five sample units are typically drawn at random from a production lot. However, in this survey, single sample units were collected from partial lots displayed at retail. In cases of positive results, these factors would have to be taken into consideration during the food safety investigations and health risk assessments.

Furthermore, imported samples were collected from available samples at retail without a requirement for the number of samples to be collected per country. In cases of positive results, unsatisfactory rates between countries are not considered to be statistically comparable.

3 Results

Pathogenic bacteria and generic *E. coli* were not detected in the majority of samples (98%) (Table 5). *E. coli* O157:H7/NM or *Shigella* spp. were not detected in any of the herb samples in this survey.

Table 5. Summary of Assessment Results of the Analysed Herb Samples

Product Origin	Number of Samples	Assessment					
		Investigative		Unsatisfactory		Satisfactory	
		Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples	Number of Samples	Percentage of Samples
Imported	816	9	1.1	8	1.0	799	97.9
Domestic	408	1	0.2	1	0.2	406	99.5
Total	**1224**	**10**	**0.8**	**9**	**0.7**	**1205**	**98.4**

A total of nine samples were found to be unsatisfactory (0.7%, 9/1224). One sample (0.08%) was unsatisfactory due to the presence of *Salmonella* spp. and the other eight samples (0.7%) were unsatisfactory due to high levels of generic *E. coli* (>1000 CFU/g). Two serotypes of *Salmonella* (*S.* Poona and *S.* Oranienburg) were identified from the isolates submitted from the one *Salmonella* positive sample (Table 7). The high levels of generic *E. coli* were found in seven imported samples and one domestic sample (Table 6). The levels of generic *E. coli* in the eight samples ranged from 1200 to 7600 CFU/g with an average level of 3062 CFU/g.

Of the nine unsatisfactory samples, eight were imported (8/816, 1.0%) and one was domestic (1/408, 0.2%). The eight unsatisfactory imported samples originated from three countries: Mexico (3/56, 5.4%), the U.S. (3/395, 0.8%), and the Dominican Republic (2/203, 1.0%). The unsatisfactory herb samples were found in five herb types: basil (3/213, 1.4%), dill (1/11, 9.0%), marjoram (1/44, 2.3%), oregano (3/63, 4.8%), and tarragon (1/67, 1.5%).

Table 6. Summary of Unsatisfactory Samples

Product Origin	Type of Herb (Country of Origin)	Reason for Unsatisfactory Assessment
Imported	Basil (Mexico)	*Salmonella* Poona & *Salmonella* Oranienburg
	Basil (Mexico)	Generic *E. coli*: 7600 CFU/g
	Basil (USA)	Generic *E. coli*: 3500 CFU/g
	Dill (USA)	Generic *E. coli*: 2000 CFU/g
	Marjoram (Mexico)	Generic *E. coli*: 1900 CFU/g
	Oregano (USA)	Generic *E. coli*: 1600 CFU/g
	Oregano (Dominican Republic)	Generic *E. coli*: 3000 CFU/g
	Tarragon (Dominican Republic)	Generic *E. coli*: 1200 CFU/g
Domestic	Oregano	Generic *E. coli*: 3700 CFU/g

All unsatisfactory samples were subject to food safety investigations and appropriate follow-up activities were conducted by the CFIA. One product recall resulted from the referral of the unsatisfactory samples to the appropriate CFIA programs and subsequent investigations.

As part of the investigations, a total of 44 follow-up samples were taken in response to the unsatisfactory results. Of these follow-up samples, *Salmonella* spp. were detected in two mint samples imported from Mexico and serotypes of *Salmonella* Montevideo, and *Salmonella* Urbana were identified from the *Salmonella* isolates. Heightened sampling and a review of importation procedures were implemented. However, it is important to note that there were no reported illnesses associated with the consumption of any of the leafy herbs during this survey and subsequent investigations.

In addition, elevated levels of generic *E. coli* (100 - 1000 CFU/g) were found in 10 samples (0.8%, 10/1224). These samples were assessed as investigative and requiring further evaluation as the *E. coli* counts were elevated but below the unsatisfactory threshold of 1000 CFU/g (Table 7). The levels of generic *E. coli* ranged from 130 to 980 CFU/g with an average level of 420 CFU/g. Evaluation of these samples resulted in no follow-up activities.

Of the 10 investigative samples, 9 samples were imported from four countries and one sample was domestically produced. These investigative samples were found in a variety

of herbs: two samples each of basil and dill; and one sample each of marjoram, mint, oregano, rosemary, sage and thyme (Table 7).

Table 7. Summary of Samples with Elevated Levels of Generic *E. coli*

Product Origin	Type of Herb (Country of Origin)	Generic *E. coli* Counts (CFU/g)
Imported	Basil (Mexico)	140
	Basil (USA)	130
	Dill (USA)	540
	Dill (USA)	280
	Marjoram (Dominican Republic)	310
	Mint (USA)	180
	Oregano (USA)	980
	Rosemary (Colombia)	460
	Thyme (USA)	540
Domestic	Sage	640

4 Discussion and Conclusion

Over ninety eight percent (98.4%) of the samples of leafy herbs tested in this survey were not contaminated with the bacterial pathogens or with the indicator bacteria used by the CFIA to assess if a potential failure in general sanitation and hygiene practices arose at some point in the production chain.

In this survey, *Salmonella* spp. were detected in one sample (0.08%) which resulted in further investigation by the CFIA as this organism is a potential source of illness, however, no reported illnesses were associated with consumption of any of the product types in this survey during the time of this study.

High or elevated levels of generic *E. coli* were detected in 1.5% of the samples. While generic *E. coli* are not disease-causing agents, their presence is used by the CFIA as an indicator that unwanted micro-organisms could be introduced during the production, processing, and marketing of these commodities. Potential reasons for the contamination could be the result of inadequate practices during production, packaging, or storage.

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. The CFIA as well as other jurisdictions such as provincial and municipal regulators do monitor the controls throughout the food production chains. 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.

The 2009/10 targeted survey found that of the 1224 leafy herb samples tested:

- Pathogenic bacteria and generic *E. coli* were not detected in the majority of samples (98.4%).
- Pathogenic bacteria *E. coli* O157:H7/NM and *Shigella* spp. were not detected in any of the samples.
- A total of nine samples were found to be unsatisfactory (0.7%). One sample (0.08%) was unsatisfactory due to the presence of *Salmonella* spp. and the other eight samples (0.7%) were unsatisfactory due to high levels of generic *E. coli* (i.e. > 1000 CFU/g).
- Elevated levels of generic *E. coli* (100 - 1000 CFU/g) were found in 0.8% (10) of the samples. These samples were assessed as investigative and requiring further evaluation as the *E. coli* counts were elevated but below the unsatisfactory threshold of 1000 CFU/g. Evaluation of these samples did not result in any follow-up activities.

The CFIA conducted follow-up activities on each of the unsatisfactory samples, including food safety investigations, health risk assessments, directed sampling, review of importation procedures, etc.

The findings of this survey indicate that contamination of leafy herbs with pathogenic micro-organisms does occur and could be a potential source of foodborne illness in Canada.

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

CI: Confidence Interval

FDA: Food and Drug Act

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

GAP: Good Agricultural Practices

GMP: Good Manufacturing Practices

HC: Health Canada

MPN: Most Probable Number

PCR: Polymerase Chain Reaction

PFGE: Pulsed Field Gel Electrophoresis

PHAC: Public Health Agency of Canada

***Salmonella* spp.**: *Salmonella* species

***Shigella* spp.**: *Shigella* species

US FDA: United States Food and Drug Administration

Appendix B: Global Foodborne Disease Outbreaks Associated with Fresh Leafy Herbs Contaminated with Bacterial Pathogens (1997-2009) *

Year	Product	Micro-organism	Country	Number of Cases	Source	Summary
2007	Basil	*Salmonella* Senftenberg	United Kingdom	32	Foodborne Pathogens and Disease, Vol 5, No 5	Imported from Israel
2007	Basil	*Salmonella* Senftenberg	Multiple states, USA	11	CDC 2007	
2006	Basil	Enterohemorrhagic *E. coli*	Denmark	250	European Food Safety Authority	*S.* Anatum also isolated from left over pasta & pesto.
2005	Parsley	*E. coli* 0157:H7	Oregon, USA	18	ProMed Oct. 25, 2005 & FSNet Oct. 31 2005	Contaminated parsley consumed in at least two restaurants.
2005	Parsley	*E. coli* 0157:H7	Washington, USA	4	CDC 2005	Parsley, house salad
2005	Parsley	*E. coli* 0157:H7	Washington, USA	2	CDC 2005	Parsley, house salad
2002	Cilantro	*Salmonella* Newport	Colorado, USA	13	CDC	
2001	Cilantro	*Salmonella* Newport	California, USA	8	CDC	
2000	Basil	*E. coli* O169:H41	Washington, USA	100		Emerging Infectious Diseases Vol. 10; No. 3, 2004
1999	Basil	*Shigella sonnei*	Multiple States, USA	10	CDC	Multistate: Connecticut, Massachusetts
1999	Cilantro	*Salmonella* Thompson	California, USA	35	CDC	
1998	Parsley	*E. coli* O6:H16	Minnesota, USA	42	Emerging Infectious Diseases 2004, 10(3) & Journal of Food Protection 2003 66(4):535-541	Parsley-red pepper mix implicated.
1998	Parsley	Enterotoxigenic	Minnesota, USA	35	J Food Protection 2003;66(4):535-541	Parsley suspected

Year	Product	Micro-organism	Country	Number of Cases	Source	Summary
1998	Parsley	Multiple Organisms	Multiple Countries	1126	J Food Protection 2003;66(4):535-541	
1998	Parsley	*Shigella boydii*	Massachusetts, USA	6	Journal of Food Protection 2003, 66(4):535-541 & JFP 68 (3):521-527	
1998	Parsley	*Shigella boydii*	Florida, USA	37	Journal of Food Protection 2003, 66(4):535-541 & JFP 68 (3):521-527	
1998	Parsley	*Shigella sonnei*	Alberta, Canada	4	Journal of Food Protection 2003, 66 (4):535-541	1,600-acre farm in Baja California, Mexico likely source
1998	Parsley	*Shigella sonnei*	Ontario, Canada	35	Morbidity and Mortality Weekly Report (MMWR) 1998, 48(14):285-9	Illness associated with attending a food fair & eating a smoked salmon & pasta dish made with fresh chopped parsley.
1998	Parsley	*Shigella sonnei*	British Columbia, Canada	13	Canada Communicable Disease Report 1999, Vol 25	Parsley from Salinas, California.
1998	Parsley	*Shigella sonnei*	California, USA	9	J Food Protection 2003;66(4):535-541	

* 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: Analytical Methods Used for Microbial Analysis

Bacterial 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
***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
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Fecal Coliforms and of *E. coli* in Foods
	MFHPB-27 (September 1997)	Enumeration of *Escherichia coli* in Foods by the Direct Plating (DP) Method

* *Compendium of Analytical Methods* (22)