Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Targeted Surveys

REPORT

2012/13 – 2013/14 Targeted Surveys

Targeted Surveys Investigating Bacterial Pathogens and generic *E. coli* in Pre-packaged Fresh-cut Ready-to-Eat Vegetables

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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 health 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 information on certain hazards in foods, identifying/characterizing new and emerging hazards, informing trend analysis, prompting/refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

In recent years, the availability of fresh-cut ready-to-eat (RTE) vegetables has increased to meet consumers' demand for convenient and healthy foods. Fresh vegetables, including fresh-cut vegetables and vegetable mixes, have been associated with several outbreaks of foodborne illnesses worldwide. Vegetables can become contaminated with pathogens during production, harvest, post-harvest handling, processing, packaging and distribution. The additional processing steps that fresh-cut vegetables go through like cutting, slicing, peeling and shredding remove or damage the protective surfaces of vegetables further increasing the possibility for microbial contamination. Since pre-packaged RTE fresh-cut vegetables are expected to be consumed raw without further preparation, the presence of pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, pre-packaged fresh-cut RTE vegetables have been selected for enhanced surveillance. Over the course of a four-year baseline study (2012/13 to 2015/16), approximately 4,500 pre-packaged fresh-cut RTE vegetables samples (with and without dressing kits) will be collected from Canadian retail locations and tested for bacterial pathogens of concern.

The main objective of these targeted surveys (2012/13 – 2013/14) was to generate baseline surveillance data on the presence and distribution of the bacterial pathogens *Campylobacter* spp., *Escherichia coli* (*E. coli*) O157:H7/NM, *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella* spp. and *Shigella* spp. and the indicator of fecal contamination generic *E. coli,* in imported and domestically produced pre-packaged fresh-cut RTE vegetables. A total of 2,679 pre-packaged fresh-cut RTE vegetable samples with and without dressing kits of both domestic and imported origin were collected. Most samples (99.7%) were assessed as satisfactory. *Campylobacter* spp., *E. coli* O157:H7/NM, *Salmonella* spp. and *Shigella* spp. were not detected in any samples. Generic *E. coli* levels were also acceptable for all samples. Only *L. monocytogenes* was detected in seven samples (0.3%). The levels detected were low

(below 5 CFU (colony forming unit)/g) in six of these samples and much higher (1,300 CFU/g) in the other sample. As a result of these findings, the Canadian Food Inspection Agency (CFIA) conducted food safety investigations and appropriate follow-up activities. Five products were recalled from the market place as a result of health risk assessments. It is important to note that there were no reported illnesses associated with the contaminated products sampled during these surveys.

These results suggest that the vast majority of pre-packaged fresh-cut RTE vegetables available in the Canadian market sampled during this survey were produced under Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs), although sporadic contamination with *L. monocytogenes* can occur.

The CFIA provides regulatory 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. In addition, 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 is targeted surveys, which are a means to establish baseline information on specific hazards in food commodities and to investigate emerging risks. Targeted surveys are one of the Agency's core surveillance strategies, which also include the National Chemical Residue Monitoring Program (NCRMP), the National Microbiological Monitoring Program (NMMP), and the Children's Food Project (CFP). The targeted surveys are complementary to other CFIA surveillance activities in that they examine foods and hazards that may not be routinely assessed in these national monitoring programs.

Targeted surveys are used to gather information on 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 possible food-hazard combinations, it is neither feasible, nor should it be necessary, to use targeted surveys to identify and quantify all hazards in all food commodities. To identify the 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 – 2013/14) mark the beginning of a four year (2012/13 to 2015/16) study on pre-packaged fresh-cut ready-to-eat (RTE) vegetables (with and without dressing kits) and represent part of the collection of approximately 4,500 samples. The study was designed to gather baseline information on the occurrence of bacterial pathogens of concern and generic *Escherichia coli* (*E. coli*) in these products 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 pre-packaged fresh-cut RTE vegetables are

encouraged to follow these international codes of practice. Other organizations such as government institutions and industry associations also develop and publish guidelines to provide guidance to food producers and manufacturers on the safe production and handling of foods. Of relevance for this survey are the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CAC/RCP 53-2003) along with Annex 1 on *Ready-to-Eat Fresh Pre-cut Fruits and Vegetables,*[1] the *Recommended International Code of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969)[2] and CFIA's *Food Safety Practices Guidance for Ready-to-Eat Fresh-Cut Vegetable Manufacturers*[3]. These codes address Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) which, when applied, control and reduce the potential for contamination of fresh-cut RTE vegetables with microbial, chemical, and physical hazards at all stages of the production from primary production to packaging.

Pre-packaged fresh-cut RTE vegetables available in the Canadian market must comply with the *Food and Drugs Act* (FDA)[4] and the *Food and Drug Regulations* (FDR)[5], 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 the safety requirements of the *Fresh Fruit and Vegetable Regulations*[6] under the *Canada Agricultural Products Act*[7]. These regulations are intended to ensure that fresh fruits and vegetables sold to consumers are safe, wholesome and properly graded, packaged and labeled. The *Fresh Fruit and Vegetable Regulations*, and the food-related portions of the FDA and FDR are enforced by the CFIA.

Targeted surveys are primarily conducted for surveillance and not for regulatory compliance purposes. However, results indicating a potential risk to public health for any samples tested under this survey will trigger food safety investigations, including activities such as follow-up sampling, inspections of facilities, and consultations with Health Canada for health risk assessments. Depending on the findings, a recall of the affected product may be warranted.

2 Survey on Pre-packaged Fresh-cut RTE Vegetables

2.1 Rationale

Fresh vegetables, including fresh-cut vegetables and vegetable mixes have been reported to be responsible for several outbreaks of foodborne illnesses worldwide. Between 2008 and 2013, at least 26 foodborne disease outbreaks were associated with vegetables (other

than leafy greens, green onions, tomatoes and sprouts, covered in separate reports) contaminated with bacterial pathogens (Appendix B). In Canada, between 2011 and 2014, there have been 24 non-outbreak related recalls of fresh-cut RTE vegetables and vegetable/salad mixes contaminated with bacterial pathogens (Appendix C).

Fresh vegetables can become contaminated at any points along the production chain, from the field to the distribution point, and become the source of contamination of fresh-cut vegetables. Processing (e.g., cutting, peeling, shredding, and packaging) and storage of fresh-cut vegetables provide additional opportunities for cross-contamination and potential for growth of bacterial pathogens. Cutting, slicing, peeling, shredding and other such processes remove or damage the protective surfaces of vegetables and increase the possibility for microbial contamination[8]. Also, cutting releases potentially nutrient-containing fluid from vegetables which promotes the growth of bacteria [1,9].

Particularly mechanical cutters and slicers can be potent sources of contamination since they usually have sites within the machines inaccessible to cleaning and disinfection, which may contain bacteria. Exposing vegetables to different types of cutting has been shown to result in a six to seven-fold increase in microbial contamination[10]. Furthermore, inappropriate temperatures and humidity levels during preparation, distribution, storage and/or retail display can also encourage the growth of bacteria on RTE fresh-cut vegetables [11, 12, 13].

Although many RTE vegetables are washed (often in chlorinated water) or otherwise disinfected to reduce contamination, this is limited to surface microflora contamination. The internalization of microbial contamination in vegetables reduces the efficacy of surface antimicrobial treatments generally used[10]. Furthermore, some microorganisms may spread from contaminated parts to uncontaminated parts during the washing process and may proliferate by taking advantage of nutrients that may be released during the washing process[8].

With consumers' interest in fresh-cut RTE vegetables increasing due to convenience and the recognized health benefits of eating produce regularly, and based on the above information highlighting the potential risks for these commodities to be contaminated with bacterial pathogens, pre-packaged fresh-cut RTE vegetables have been selected for targeted surveillance. The overall objective of this targeted surveillance is to gather baseline information on the occurrence of various pathogens of concern and generic *E. coli*, an indicator of fecal contamination, in pre-packaged fresh-cut RTE vegetables available to Canadians at retail.

These targeted surveys (2012/13 – 2013/14) mark the beginning of a four year baseline study (2012/13 to 2015/16) and focused on investigating the presence and distribution of bacterial pathogens, as well as the presence, distribution, and levels of generic *E. coli* (as an indicator of fecal contamination) in imported and domestic pre-packaged fresh-cut RTE vegetables, with and without dressing kits.

2.2 Targeted Microorganisms

2.2.1 Bacterial Pathogens of Concern

Salmonella and *E. coli* O157:H7 are found naturally in the intestines of animals, such as poultry and cattle respectively[14]. Most outbreaks associated with these bacterial pathogens are linked to the consumption of contaminated foods of animal origin (e.g., chicken and beef burger). However, fresh fruits and vegetables have emerged as significant sources of illnesses caused by these bacterial pathogens in the last decade[15]. Fruits and vegetables can become contaminated with these bacterial pathogens in the field by improperly composted manure, contaminated water, wildlife feces, and/or poor hygienic practices of farm workers[16].

Humans are the only host of the bacterial pathogen *Shigella*. Food contaminated by infected food handlers with poor personal hygiene, and water contaminated with human feces are the most common causes of shigellosis. Shigellosis has been known to be associated with the consumption of contaminated fruits, vegetables, shellfish, and chicken[15].

Similar to *Salmonella* and *E. coli* O157:H7, the 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.[17] and Canada[18]. Raw poultry and unpasteurized (raw) milk are major sources of contaminated food. However, vegetables were also found, sporadically, to be contaminated with *Campylobacter*[15].

Listeria monocytogenes (L. monocytogenes) is widely distributed in the environment and has been isolated in a wide variety of foods, including raw vegetables. Likely sources of vegetable contamination include soil, contaminated irrigation water or wash water, decaying vegetation, as well as the processing and packaging environment. Compared to other bacterial pathogens, *L. monocytogenes* has a wide range of growth temperatures (-0.4 to 45ºC) that includes the typical refrigeration temperature of 4°C. Contaminated fresh-cut vegetables that are capable of supporting limited growth of the bacteria at refrigeration temperatures have been implicated in a few outbreaks of foodborne listeriosis[19].

2.2.2 Generic *E. coli* as an Indicator of Fecal Contamination

Typically, *E. coli* 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 [20]. 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 are an indication that contamination has occurred at some point between primary production and the time of sale.

2.3 Sample Collection

Pre-packaged fresh-cut RTE vegetable samples consisted of peeled, sliced, chopped or shredded beets, broccoli, carrots, cauliflower, celeries, green beans, leeks, mushrooms, onions, peppers, squashes, sugar snap peas, and a variety of slaws, vegetable mixes and salad mixes, with or without dressing kits. Note that the kit contents (dip, salad dressing, croutons, cheese, etc.) were required to be provided as a separate side in the package and not be already mixed with the vegetables (as the scope of these surveys did not include prepared salads).

All samples were collected from national chain and local/regional grocery stores and other conventional retail and natural and health food stores located in various cities across Canada. The number of samples collected in the various regions was based on the relative proportion of the population in the respective regions and were collected during the 2012/13 and 2013/14 fiscal years (April 1, 2012 to March 31, 2014). For samples without kits, domestic samples were mainly collected during the late summer months in this survey while imported samples were collected primarily in the fall, winter, and spring months. However, samples with kits were collected more or less equally throughout the year regardless of origin.

For this survey, a sample consisted of a single sample unit (e.g., individual consumer-size bundle(s) from a single lot) with a total weight of at least 150g. This sampling approach is common for surveys conducted at retail and is also used by other federal partners such as the Public Health Agency of Canada (PHAC) for the retail component of their FoodNet Surveys[21]. Collected samples were required to be shipped under conditions that limit the growth of microorganisms during transit. Samples were declared “unfit” for

analysis if there were issues regarding the conditions in which they were handled or shipped.

2.4 Analytical Methods and Assessment Guidelines

Samples were analysed using the analytical methods as published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods[22] (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 pre-packaged fresh-cut RTE vegetables. For the samples packaged with dressing kits, only the vegetable portion of the samples was analysed (i.e., the dressing kit was not included in the analysis).

The assessment criteria used for this survey (Table 1) are based on the principles of 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], as well as Health Canada's *Policy on Listeria monocytogenes in Ready-to-Eat Foods (2011)*[19].

Table 1. Assessment Criteria for *Campylobacter*, *E. coli* O157:H7/NM, *Salmonella* and *Shigella* in Fresh-cut vegetables (with and without kits)

Bacterial Analysis* **(Method Identification Number)**	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
Campylobacter spp** (MFLP-46 modified)	Absent in 25g	Present in 25g
***E. coli* O157:H7/NM** (MFLP-30 and MFLP-80 if required for confirmation)	Absent in 25g	Present in 25g
Salmonella* spp.* (MFLP-29 modified and MFHPB-20 if required for confirmation)	Absent in 25g	Present in 25g
Shigella* spp.* (MFLP-26 and MFLP-25 if required for confirmation)	Absent in 25g	Present in 25g

* Compendium of Analytical Methods[22]

** 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* and *L. monocytogenes* in Fresh-cut vegetables (with and without kits)

Bacterial Analysis* **(Method Identification Number)**	**Assessment Criteria**		
	Satisfactory	**Investigative**	**Unsatisfactory**
Generic *E. coli* (MFHPB-19 & 27)**	≤ 100 /g	100 < x ≤ 1000 /g	> 1000 /g
L. monocytogenes (MFLP-28, MFHPB-30 and MFLP-74 if required for enumeration)	Not Detected in 25 g	Detected and ≤ 100 CFU/g	> 100 CFU/g

* Compendium of Analytical Methods [22]
** Unit for MFHPB-19 method: MPN/g, for MFHPB-27 method: CFU/g.

Based on the current regulatory standards and microbiology testing criteria, results in these surveys are assessed as "satisfactory," "unsatisfactory" or "investigative". Samples assessed as unsatisfactory are subject to follow-up actions, such as directed follow-up sampling, establishment inspection, health risk assessment, and/or product action (e.g., product recall).

Samples assessed as investigative for generic *E. coli* required some form of follow-up activity, such as further sampling to verify the levels of generic *E. coli* in the products in question.

Samples assessed as investigative for *L. monocytogenes* also required some form of follow-up activities, which included the evaluation of the product's shelf-life and physico-chemical parameters such as pH if applicable, to determine the potential food safety risk associated with the product in question. Typically for fresh-cut vegetables, the results were considered acceptable if the stated shelf-life of the product was five days or less, and unacceptable if the stated shelf-life was more than five days.

2.5 Limitations

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.

3 Results

3.1 Sample Distribution

A total of 2,679 pre-packaged fresh-cut RTE vegetable samples were collected, including 870 samples packaged with dressing kits and 1,809 samples without kits (Table 3). Samples packaged with dressing kits consisted at 89.9% of mixed vegetables (e.g., vegetable party trays, vegetable snacks/sticks). Samples without kits consisted mainly of mushrooms (27.3%), mixed vegetables (26.0%), slaws (22.2%), carrots (11.2%) and broccoli (7.6%) (Table 3).

Table 3. Distribution of Pre-packaged Fresh-cut RTE Vegetables (with and without dressing kits) by Product Type.

Product Type	Pre-packaged Fresh-cut RTE Vegetables	
	Samples without kits	Samples with kits
Beets	1 (0.1%)	-
Broccoli	138 (7.6%)	4 (0.5%)
Carrots	203 (11.2%)	18 (2.1%)
Cauliflower	20 (1.1%)	-
Celery	19 (1.1%)	3 (0.3%)
Green Beans	3 (0.2%)	-
Leeks	2 (0.1%)	-
Mixed Vegetables	471(26.0%)	782 (89.9%)
Mushrooms	494 (27.3%)	1 (0.1%)
Onions	3 (0.2%)	-
Peppers	34 (1.9%)	1 (0.1%)
Salad Mix	13 (0.7%)	47 (5.4%)
Slaws	401 (22.2%)	11 (1.3%)
Squash	6 (0.3%)	-
Sugar Snap Peas	1 (0.1%)	3 (0.3%)
Total = 2,679	**1809**	**870**

As seen in Table 4, a large percentage of the samples packaged without dressing kits were from Canada (48.7%) and the U.S. (48.0%). In contrast, the country of origin of a large number of samples packaged with dressing kits could not be identified (40.6%). Most of these samples were vegetable mixes, and as such could have been prepared with vegetables from different origins, making the labelling of these products difficult. The rest of the samples packaged with kits were from Canada (32.1%) and imported (27.4%), mostly from the United States.

Table 4. Distribution of Pre-packaged Fresh-cut RTE Vegetables (with and without salad kits) by Country of Origin.

Country of Origin	Pre-packaged Fresh-cut RTE Vegetables		
	Samples without kits	Samples with kits	Total
Canada	881 (48.7)	279 (32.1%)	1,160 (43.3%)
Imported	875 (48.4%)	238 (27.4%)	1,113 (41.5%)
Guatemala	1 (0.1%)	-	1 (0.0%)
Mexico	5 (0.3%)	4 (0.5%)	9 (0.3%)
United States	869 (48.0%)	234 (26.9%)	1,103 (41.2%)
Unknown	53 (2.9%)	353 (40.6%)	406 (15.2%)
Total	**1,809 (100%)**	**870 (100%)**	**2,679 (100%)**

3.2 Assessment Results

A total of 2,679 pre-packaged fresh-cut RTE vegetable samples were analyzed for *Campylobacter* spp., *Salmonella* spp., *E. coli* O157:H7/NM, *Shigella* spp., *L. monocytogenes* and generic *E. coli*.

All samples of pre-packaged fresh-cut RTE vegetables with dressing kits were satisfactory, as no pathogens were detected in any of these samples and levels of generic *E. coli* were always below levels of concern (Table 5).

Table 5. Summary of Results of pre-packaged fresh-cut RTE Vegetables

Product Type	Origin	Number of Samples	Assessment		
			Unsatisfactory	Investigative	Satisfactory
Without dressing kits	Domestic	881	0	5	876
	Imported	875	1	1	873
	Unknown	53	0	0	53
With dressing kits	Domestic	279	0	0	279
	Imported	238	0	0	238
	Unknown	353	0	0	353
Total		**2,679 (100%)**	**1 (0.3%)**	**6**	**2,672 (99.7%)**

For the pre-packaged fresh-cut RTE vegetables without dressing kits, six samples were assessed as investigative due to the detection of low levels of *L. monocytogenes* (<100 CFU/g) and one sample was assessed as unsatisfactory due to high levels (> 1,000 CFU/g) of *L. monocytogenes* (Table 6). As a result of these findings, the CFIA

conducted food safety investigations and appropriate follow-up activities. Two of the products with investigative assessment were deemed acceptable following further evaluation as they had a stated shelf-life of less than five days. The five other products were recalled from the market place after subsequent evaluations and health risk assessments.

Table 6 Summary of Samples Positive for *L. monocytogenes*

Product Type/ Country of Origin	**Levels Detected / Results of Further Evaluations**
Diced onion, imported from the U.S.	< 5 CFU/g / unacceptable, recalled
Fresh-cut Vegetable mix, domestic (shelf-life of less than five days)	< 5 CFU/g / acceptable
Sliced crimini mushrooms, domestic	1,300 CFU/g / unacceptable, recalled
Fresh-cut lobster mushrooms, domestic	< 5 CFU/g / unacceptable, recalled
Fresh-sliced crimini mushrooms, domestic	< 5 CFU/g / unacceptable, recalled
Fresh-cut cauliflower, domestic (shelf-life of less than five days)	< 5 CFU/g / acceptable
Stir-fry style vegetables, imported from the U.S.	< 5 CFU/g / unacceptable, recalled

4 Discussion and Conclusion

In the 2012/13 and 2013/14 surveys, *Campylobacter* spp., *Salmonella* spp., *E. coli* O157:H7/NM and *Shigella* spp. were not detected in any of 2,679 samples of pre-packaged fresh-cut RTE vegetables collected (with and without dressing kits) and generic *E. coli* levels were also acceptable in all samples.

L. monocytogenes was detected in seven pre-packaged fresh-cut RTE vegetable samples (0.3%). As a result of these findings, the CFIA conducted appropriate follow-up activities including food safety investigations, directed samplings, health risk assessments in consultation with Health Canada and the recall of the affected products that were still available in the marketplace. It is important to note that there were no reported illnesses associated with the consumption of the *L. monocytogenes* contaminated products during this survey.

L. monocytogenes is commonly found in soil and water, and is usually known to contaminate foods of animal origin as animals carry *L. monocytogenes* without signs of illness[24]. However, there has been increasing association of *L. monocytogenes* with fresh and fresh-cut vegetables and fruits and it has been shown that *L. monocytogenes* can actively proliferate on several vegetables (e.g., celery, asparagus and broccoli) [12]. One of the earliest listeriosis outbreaks linked to fresh-cut vegetables occurred in Canada in 1981, where coleslaw was identified as the source[8]. Another recent outbreak occurred in 2010 in the US, where diced celery was identified as the source[9]. Also worth noting is that, out of the 24 Canadian non-outbreak related recalls of fresh-cut vegetables and salads, 20 were recalled due to the presence of *L. monocytogenes* (Appendix C).

The overall finding of this survey suggests that the vast majority of pre-packaged fresh-cut vegetables available in the Canadian market are produced and handled under acceptable GAPs and GMPs. However, contamination of pre-packaged fresh-cut RTE vegetables with *L. monocytogenes* can occur sporadically, which may represent a food safety risk for high-risk population groups (e.g., pregnant women, older adults, the immunocompromised).

While the food industry and retail sectors are ultimately responsible for the food they produce and sell in Canada, and individual consumers are responsible for the safe handling of the food they have in their possession, the CFIA regulates the food industry, provides oversight and promotes safe handling of foods throughout the food production chain. The CFIA will continue its surveillance and inform stakeholders of its findings.

5 Acknowledgement

We would like to express our sincere thanks to Judy D. Greig, PHAC for providing information on outbreaks (Appendix B).

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Appendix A: List of Acronyms

CFIA: Canadian Food Inspection Agency

CFP: Children's Food Project

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

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

L. monocytogenes: *Listeria monocytogenes*

MPN: Most Probable Number

NCRMP: National Chemical Residues Monitoring Program

NMMP: National Microbiology Monitoring Program

PCR: Polymerase Chain Reaction

PFGE: Pulsed field gel electrophoresis

PHAC: Public Health Agency of Canada

RTE: Ready-to-eat

spp.: species

U.S. United States

WHO: World Health Organization

°C: Degree Celsius

g: gram

Appendix B: Global Foodborne Disease Outbreaks Associated with Whole and Fresh-cut Vegetables (Other than Leafy Greens, Green Onions, Tomatoes and Sprouts) Contaminated with Bacterial Pathogens (2008 - 2014)

Year	Country	Microorganism	Serogroup/ Subgroup	Vehicle	Cases	Number Hospitalized	Source
2008	United Kingdom	*Salmonella enterica*	Typhimurium	Salad vegetables	13		Health Protection Report
2008	United States	*Salmonella enterica*	Saintpaul	Peppers	1500	315	New England Journal of Medicine
2008	Canada	*Escherichia coli*	O121	Spanish onions	224	24	North Bay and District Health
2008	United States	*Escherichia coli*	O157	Pre-packaged salad	6	4	CDC line list 2008
2008	United States	*Salmonella enterica*	Braenderup	Salad	12	5	CDC line list 2008
2009	Norway	*Shigella sonnei*		Sugar snap peas	23	3	Eurosurveillance 2009
2009	Sweden	*Shigella dysenteriae*		Sugar snap peas	47		Eurosurveillance 2009
2009	Lithuania	*Salmonella enterica*	Enteritidis	Peas	62	39	
2009	United States	*Salmonella enterica*	Miami	Salad	9	3	CDC line list 2009

Year	Country	Microorganism	Serogroup/ Subgroup	Vehicle	Cases	Number Hospitalized	Source
2010	United States	*Listeria monocytogenes*		Celery	10		Clin Infect Dis. 2012
2010	France	*Salmonella*		Vegetables	2	2	European line list 2010
2010	France	*Salmonella enterica*	Typhimurium	Vegetables	5	1	European line list 2010
2010	Germany	*Staphylococcus*		Vegetables	24		European line list 2010
2010	United Kingdom	*Escherichia coli*	O157	Leeks and Potatoes	250	74	Health Prot. Report 2011.
2010	United States	*Escherichia coli*	O157	Vegetables	4		State Health Dept.
2010	Australia	*Salmonella enterica*	Typhimurium	Salad	47	5	Comm. Dis. Intell.
2010	Finland	*Bacillus cereus*		Salad	2		European line list 2010
2010	United Kingdom	*Salmonella enterica*	Java	Salad	136		Eurosurveillance, 2011
2011	Denmark	*Escherichia coli*	O27	Sugar snap peas	2		EU 2011
2011	Japan	*Salmonella*		Broccoli salads	1500		Hokkaido Prefectural Gov.
2011	Russia	*Yersinia pseudotuberculosis*		Vegetables	9		Rospotrebnadzor

Year	Country	Microorganism	Serogroup/ Subgroup	Vehicle	Cases	Number Hospitalized	Source
2011	United Kingdom	*Listeria monocytogenes*		Pre-packed sandwiches and salads	3		Eurosurveillance, 2011
2011	United States	*Salmonella enterica*	Typhimurium	Salad	15		Kane County Health Dept.
2011	United States	*Escherichia coli*		Salad	33		St. Louis County Health Dept.
2013	United States	*Salmonella enterica*	Saintpaul	Cucumbers	84	17	CDC
2013	United States	*Escherichia coli*	O157	Salad	33	7	CDC
2014	United Kingdom	*Escherichia coli*	O96	Cucumbers and Lettuce	50		Health protection Report 8 (31) 2014

Appendix C: Canadian Recalls Associated with Fresh-cut Vegetables (Other than Leafy Greens, Green Onions, Tomatoes and Sprouts) Contaminated with Bacterial Pathogens (2011 – 2014)

Year of Recall	Month of Recall	Microorganism	Vehicle	Country of Origin
2011	May	*Salmonella*	Vegetable platters	Unidentified
2011	June	*Listeria monocytogenes*	Salad	United States
2011	December	*Listeria monocytogenes*	Mushrooms	Canada
2011	December	*Listeria monocytogenes*	Salad	Unidentified
2012	May	*Listeria monocytogenes*	Salad	United States
2012	May	*Listeria monocytogenes*	Fresh diced red onions	United States
2012	May	*Listeria monocytogenes*	Salad	United States
2012	May	*Listeria monocytogenes*	Salad	United States
2012	May	*Listeria monocytogenes*	Salad	United States
2012	May	*Listeria monocytogenes*	Salad	United States
2012	July	*Listeria monocytogenes*	Fresh shelled peas	Unidentified
2012	July	*Listeria monocytogenes*	Organic Italian blend	United States
2012	July	*Listeria monocytogenes*	Fresh diced red onions, yellow onions and celery	United States
2012	July	*Listeria monocytogenes*	Fresh diced red onions, yellow onions and celery	United States
2012	August	*Listeria monocytogenes*	Sliced white mushrooms	Canada
2012	August	*Listeria monocytogenes*	Mixed vegetables and mushrooms	Canada
2012	August	*Listeria monocytogenes*	Sliced white mushrooms and crimini	Canada

Year of Recall	Month of Recall	Microorganism	Vehicle	Country of Origin
2012	August	*Listeria monocytogenes*	Sliced white mushrooms and crimini	Canada
2012	September	*Listeria monocytogenes*	Sliced lobster mushrooms	Canada
2012	September	*Listeria monocytogenes*	Sliced crimini	Canada
2012	October	*Salmonella enterica* Bredney	Salad kit with peanuts	Unidentified
2013	May	*Microbiological-other*	Cut green beans	Unidentified
2014	February	*Salmonella*	Salad with alfalfa sprouts	Canada
2014	March	*Listeria monocytogenes*	Italian blend salad mix	Unidentified

Source: CFIA Recalls

Appendix D: Analytical Methods Used for Microbial Analysis

Microbial Analysis	Method Identification Number (Date Issued)	Title of Method[1]
E. coli O157:H7/NM	MFLP-30	The Dupont Qualicon Bax® System Method for the Detection of *E. coli* O157:H7 in Raw Beef and Fruit Juice
	MFLP-80 (March 2008)	Isolation of *E. coli* O157:H7 or NM in Foods
Campylobacter spp.	MFLP-46 (March 2002, Modified[2])	Isolation of Thermophilic *Campylobacter* from Foods
L. monocytogenes	MFLP 28 (November 2011)	The Qualicon Bax® System Method for the Detection of *Listeria monocytogenes* in a Variety of Food
	MFHPB-30 (February 2011)	Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples
	MFLP-74 (February 2011)	Enumeration of *Listeria monocytogenes* in Food
Salmonella spp.	MFLP-29[3] (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
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in Foods
	MFHPB-27 (October 2012)	Enumeration of *Escherichia coli* in Foods by the Direct Plating (DP) Method

[1] *Compendium of Analytical Methods*[23].

[2] MFLP-46 was performed with the following modification to include wash with peptone water to collect *Campylobacter* from the samples, followed by enrichment.
[3] 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.