Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2010/2011 Targeted Surveys

Targeted Survey Investigating Viral Pathogens in Leafy Vegetables and Green Onions

RDIMS#3937470

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, viruses have been increasingly recognized as a major cause of foodborne illnesses. Norovirus (NoV) and hepatitis A virus (HAV) are the most frequently reported human enteric viruses involved in foodborne illnesses. An expert committee of the FAO/WHO (Food and Agriculture Organization of the United Nations and World Health Organization) recently determined that NoV and HAV in fresh produce were one of the virus-commodity combinations of highest priority in terms of food safety. According to foodborne outbreak information provided by the Public Health Agency of Canada for the period between 1998 and 2010, NoV accounted for approximately one third of outbreaks associated with leafy vegetables worldwide, while HAV was the predominant pathogen in outbreaks associated with green onions. Leafy vegetables and green onions can become contaminated with enteric viruses through contact with human sewage or infected workers during primary production, harvest, post-harvest handling, processing, packaging, and distribution. Unlike bacteria, human enteric viruses cannot multiply in food, as they need to enter living human cells to replicate. However, they can remain viable in vegetables for extended periods of time, and may cause illness if ingested.

Considering the factors mentioned above and their relevance to Canadians, leafy vegetables and green onions have been selected for enhanced surveillance under the FSAP. Between 2008/09 - 2012/13, about 5,000 samples of fresh fruits and vegetables were collected from Canadian retail locations and tested for the presence of viral pathogens of concern.

The main objective of the 2010/11 targeted survey was to generate baseline surveillance data on viral pathogens NoV and HAV for imported and domestically produced leafy vegetables and green onions available in the Canadian market. In total, 1112 samples of pre-packaged leafy vegetables and 549 samples of green onions were collected and analyzed. HAV was not detected in any of the samples tested, while NoV was detected in 25 samples of leafy vegetables (2.2%) and three samples of green onions (0.5%). Positive results indicate that the products came in contact with the virus at some point of the production and distribution chain, suggesting that Good Agricultural Practices (GAPs) or Good Manufacturing Practices (GMPs) were not followed or appropriately implemented. Immediate follow-up activities were not possible as the types of products examined during this survey had a very short shelf-life and were no longer on the market by the time the results were confirmed. No NoV or HAV outbreaks associated with the

consumption of these products were reported during this survey. As current methods for virus detection are molecular-based assays that do not discriminate live, infectious viruses, from dead viruses, it is not possible to determine whether the positive samples were capable of causing illness based on laboratory results alone. It is important to note that food virology is a fairly emerging field, and that there are currently no internationally recognized assessment criteria and harmonized analytical methods for the detection of viruses in fresh produce.

The CFIA regulates and provides oversight to 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 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 system for food, health and consumer products. 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 of occurrence of these risks, improve import and domestic food controls, and identify food importers and manufacturers.

Within the FSAP, there are 12 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

Targeted surveys are used to gather information regarding the potential occurrence of hazards in food commodities. The microbiological targeted surveys aim to establish baseline data on priority and/or emerging microbiological hazards in targeted commodities, primarily fresh fruits and vegetables and imported food ingredients. A statistically significant number of samples were collected over five years to allow for seasonal and/or production variations. This work differs from regular CFIA microbiological monitoring activities which test samples of a broad range of commodities for multiple hazards to determine the compliance of defined lots with established microbial standards or guidelines for regulatory purposes.

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, a group of Canadian federal, provincial and territorial subject matter experts in the area of food safety (3).

This survey (2010/11) represents part of the collection of over 5,000 fresh fruits and vegetable samples over five years (2008/09 – 2012/13) of targeted surveys, and was designed to gather baseline information on the occurrence of viral pathogens of concern in fresh fruits and vegetables.

1.3 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 FAO/WHO Codex Alimentarius Commission. Producers of fresh fruits and vegetables are encouraged to follow the international codes of practice and guidelines. Of relevance for this survey are the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CAC/RCP 53-2003) (4) and the *Recommended International Codes of Practice-General Principles of Food Hygiene* (CAC/RCP 1-1969) (5). 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 production of fresh fruits and vegetables, from primary production to packaging. Additionally to these codes, the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food* (including fresh produce, Annex II) (CAC/GL 7902012) (6) were recently drafted to propose ways to prevent fresh produce from becoming contaminated by viruses during production.

Fresh fruits and vegetables available in the Canadian market must comply with the *Food and Drugs Act* (FDA) (7) and the *Food and Drug Regulations* (FDR) (8), 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* (9) under the *Canada Agricultural Products Act* (10). 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.

The FSAP targeted surveys are primarily conducted for surveillance and not for regulatory compliance verification purposes. Presently, the CFIA does not test foods for viruses under its national microbiological monitoring program. This is largely due to the

absence of internationally recognized standards and harmonized analytical methods for the detection of viruses in foods.

2 Survey on Viruses in Fresh Leafy Vegetables and Green Onions

2.1 Rationale

Leafy vegetables and green onions have been associated with several outbreaks of foodborne illnesses worldwide. Many of these outbreaks were associated with viruses. Outbreak information provided by the Public Health Agency of Canada (PHAC) indicates that, from 1998 to 2010, leafy vegetables contaminated with microbial pathogens were implicated in 93 outbreaks worldwide (Appendix B), of which approximately one third (30, 32.3%) were due to viruses (Appendix C). Of these virus-associated outbreaks, 27 (90.0%) were caused by NoV. In contrast, from 1996 to 2010, green onions contaminated with microbial pathogens were implicated in eight outbreaks worldwide, of which four were caused by HAV and one by NoV (Appendix D).

Produce, such as leafy vegetables and green onions, can become contaminated with viruses pathogenic to humans during production, harvest, post-harvest handling, processing, packaging, and distribution (11). The main source of food contamination with NoV is feces and vomit from infected people. Therefore, produce can become contaminated in the field by the use of irrigation water contaminated by human sewage. Leafy vegetables and green onions require extensive handling during harvesting and packaging, and can therefore become contaminated by infected handlers. During processing, water soiled with human feces or vomitus that is used for produce rinsing, cooling and icing also represents a potential source of virus introduction. Contamination with HAV is particularly of concern in most developing countries where infection with this virus is endemic (11). There are limited effective treatments to eliminate viruses from fresh produce. Although viruses can be killed by proper cooking, their presence in fresh produce eaten raw creates a potential food safety risk.

An expert committee of the FAO/WHO recently determined that NoV and HAV in fresh produce, along with shellfish and prepared food, were the virus-commodity combinations of highest priority in terms of food safety. This determination was based on current knowledge of foodborne viral diseases (e.g., incidence, severity and potential threat to public health), deemed limited due to under-reporting and the lack of specific surveillance systems worldwide for this type of illnesses (11).

Based on the above information and the Food Safety Science Committee's recommendations (3), fresh leafy vegetables and green onions have been selected as

priority groups of virus-commodity combination for targeted surveillance under the FSAP. The overall objective is to gather preliminary baseline information on the occurrence of viral pathogens of concern in leafy vegetables and green onions available to Canadians at retail.

2.2 Targeted Viral Pathogens of Concern

NoV and HAV are the two most common foodborne enteric viruses. NoV is considered to be the leading cause of domestically acquired foodborne illnesses in the U.S. (12) and Canada (13). There are currently five recognized NoV genogroups (GI to GV); Genotypes I and II are known to be responsible for most human illnesses (14). Although the incidence of HAV foodborne illness is much lower than NoV, HAV infection can cause severe symptoms and/or outcomes. Generally, NoV causes acute gastroenteritis without long term effects. HAV causes hepatitis A, an infectious liver disease that is generally self-limiting, but with possible severe outcomes (e.g., fulminant hepatitis, reported in less than 1-1.5% of cases) (14).

Unlike bacterial pathogens, viral pathogens do not multiply in food since they need to enter living cells to replicate (11). They are however more environmentally resistant than many bacteria and can remain viable in foods for a very long time (11). Vegetables (leafy greens, herbs, and green onions), fruits (berries), and RTE-foods (salad, sandwiches), have been implicated in NoV and HAV associated foodborne outbreaks (11).

2.3 Sample Collection

Pre-packaged leafy vegetables samples and bunches of green onion samples were collected for this survey. Leafy vegetable samples consisted of arugula, escarole, endive, chicory, lettuces (e.g., head lettuce, leaf lettuce), spinach, Swiss-chard, watercress, and baby varieties of the above.

All samples were collected from national chain and local/regional grocery stores, other conventional retail and natural 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. Samples were collected between April 2010 and March 2011. Domestic samples were collected during the summer months (June-September). Imported samples were collected primarily in the fall, winter, and spring months. Samples that were labeled as organic at retail were identified as “organic” in this survey. 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 total weight of at least 200 g. 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 (15). If issues or questions arose about the conditions in which the sample was shipped, the sample was declared unfit for analysis.

2.4 Sample Distribution

As per the survey design, a total of 1661 samples, including 549 green onion samples and 1112 leafy vegetable samples were collected and analyzed for NoV and HAV (Table 1).

Table 1 Sample Distribution by Product Origin and Production Practices

Vegetable group	Product Origin	Production Practice	Number (Percentage) of Samples
Green onions	Imported	Conventional	167 (30.4%)
		Organic	147 (26.8%)
	Domestic	Conventional	179 (32.6%)
		Organic	56 (10.2%)
Subtotal			**549 (100%)**
Leafy vegetables	Imported	Conventional	669 (60.1%)
	Domestic	Conventional	443 (39.9%)
Subtotal			**1112 (100%)**

2.4.1 Sample Distribution by Country of Origin

All domestic samples (Table 1) were grown and collected in various provinces across Canada. Most imported green onion samples originated from Mexico (246 samples, 78.3%). The rest of the green onion samples were from the USA (51, 16.2%), China (1, 0.3%) and unidentified countries (16, 5.1%). The majority of imported leafy vegetable samples (98.2%) were from the U.S. (657 samples). The rest of the imported leafy vegetable samples were from Dominican Republic (4, 0.6%), Mexico (7, 1.0%) and an unidentified country (1, 0.1%).

2.4.2 Sample Distribution by Product Type

Leafy vegetable samples consisted of many product types as presented in Table 2.

Table 2 Pre-packaged Leafy Vegetable Samples by Product Type

Product Type	Number of Samples	Percentage (%)
Arugula	**50**	**4.5**
Head Lettuce (whole)	18	
Leafy Lettuce (whole):	113	
- *Romaine Lettuce*	*107*	
- *Other leafy lettuce*	*6*	
Lettuce, whole - not specified	1	
Lettuce, whole (subtotal)	**132**	**11.9**
Head Lettuce (fresh-cut)	11	
Leafy Lettuce (fresh-cut):	141	
- *Romaine lettuce*	*- 120*	
- *Other leafy lettuce*	*- 21*	
Lettuce mix	30	
Salad blend (lettuce base)	110	
Lettuce, fresh-cut -not specified	37	
Lettuce, fresh-cut (subtotal)	**329**	**29.6**
Spinach	**371**	**33.4**
Spring mix/Field greens	**210**	**18.9**
Swiss chard	**11**	**9.9**
Other*	**9**	**0.8**
Total	**1112**	**100**

* The product type was not specified.

2.5 Methods Details

The samples were analyzed for Hepatitis A Virus and Norovirus (GI and GII) using modified versions of methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods* (16) (Appendix E). Samples were first screened by reverse-transcriptase Polymerase Chain Reaction (RT-PCR). Samples that screened positive by RT-PCR were further characterized by cloning and sequencing to confirm the presence of the targeted virus. Confirmed positive results were re-

analyzed by real-time RT-quantitative PCR (RT-qPCR) to estimate the number of viral genomic copies. Results were reported as "detected" when the virus' genetic material was detected and confirmed, and as "not detected" when it was either not detected or not confirmed.

The above-mentioned methods are based on the Polymerase Chain Reaction (PCR) technology, which is used to identify pathogens by detecting a specific fragment of their genetic material. It is important to note that these PCR-based methods do not discriminate live from dead organisms. As opposed to bacterial pathogens, enteric viruses such as HAV and NoV cannot be cultured *in vitro*. Therefore, the viability of these viruses found in food samples and the potential for infection cannot be confirmed by conventional cultural methods.

2.6 Limitations

Food virology is a fairly emerging field as compared to food bacteriology. Currently, there are no internationally recognized assessment criteria for viruses in fresh produce. The only assays available for the detection and quantification of human enteric viruses NoV and HAV are molecular-based methods, which do not differentiate live (i.e., infectious) from dead viruses. This means that a food found positive for one of these viruses is not necessarily capable of causing illness. It is therefore difficult to determine the immediate health significance of a positive result without supporting epidemiological evidence linking the food to clinical cases. Furthermore, due to the perishable nature of fresh produce, the samples tested have usually well passed their shelf-life by the time the analysis is completed, preventing the possibility of any immediate follow-up activities. The poor sensitivity of the current methods, mainly due to several challenges associated with the extraction of viruses from foods, must also be kept in mind when considering the prevalence levels obtained through this survey.

This survey was designed to gather baseline information on two common viral pathogens (i.e., NoV and HAV) in foods available at retail. Given the seasonality as well as the varying channels of commerce, the origin of the products can change dramatically from one season to the next. As such, there is an insufficient number of samples in this report to carry out a detailed analysis of the results based on country of origin.

3 Results

A total of 549 bulk green onion samples were analyzed for HAV and NoV. HAV was not detected in any of the samples tested. NoV was not detected either in themajority of the samples (99.5%). NoV GI was detected in three imported green onion samples (0.5%), including two conventional and one organic sample (Table 3).

Table 3. Summary of Results for Green Onion Samples Analyzed for HAV and NoV (GI & GII)

Product Origin	Production Practice	Number of Samples	HAV		NoV	
			Detected in 25 g	Not detected in 25 g	Detected in 25 g	Not detected in 25 g
Imported	Conventional	167	0	167	2	165
	Organic	147	0	147	1	144
Domestic	Conventional	179	0	179	0	178
	Organic	56	0	56	0	56
Total		**549 (100%)**	**0 (0%)**	**549 (100%)**	**3 (0.5%)**	**546 (99.5%)**

A total of 1112 pre-packaged leafy vegetable samples were analyzed for HAV and NoV (Table 4). HAV was not detected in any of the samples tested. NoV was not detected either in the majority of the samples (97.8%). NoV was detected in a total of 25 leafy vegetable samples (2.2%), including 20 imported and five domestic samples (Table 4). Of the NoV positive samples, NoV GI was detected in 24 samples and NoV GII was detected in one sample.

Table 4 Summary of Results for Leafy Vegetable Samples Analyzed for HAV and NoV (GI & GII)

Product Origin	Number of Samples	HAV		NoV (GI and GII)	
		Detected in 25 g	Not detected in 25 g	Detected in 25 g	Not detected in 25 g
Imported	669	0	669	20	649
Domestic	443	0	443	5	438
Total	**1112**	**0 (0%)**	**1112 (100%)**	**25* (2.2%)**	**1087 (97.8%)**

* NoV GII was detected in one sample of baby spinach from Canada. All the other samples were positive for NoV GI.

Molecular-based assays (RT-qPCR) were performed to estimate the number of virus copies in the green onion samples (Table 5) and the leafy vegetable samples (Table 6) that tested positive for NoV GI. The estimated number of virus copies ranged from 18 to 30,724 per 25 grams of product in the green onion samples and 6 to 50, 660 particles per 25 g of product in the leafy vegetable samples. No enumeration was possible on the NoV GII positive sample (domestically produced baby spinach).

Table 5. Summary of Enumeration Results for Norovirus Detected in Green Onion Samples

Product Origin	Product Type/ Country of Origin	Numbers of NoV (GI) copies/25 g
Imported	Conventional/Mexico	30,724
	Conventional/Mexico	466
	Organic/Mexico	18

Table 6 Summary of Enumeration Results for Norovirus GI detected in Leafy Vegetable Samples

Product Origin	Product Type/ Country of Origin	Numbers of NoV GI copies/25 g
Imported	Caesar Salad (romaine lettuce base)/ US	50,660
	Leafy lettuce, Romaine (fresh-cut)/ US	11,144
	Salad blend (iceberg & romaine lettuce)/ US	2,628
	Lettuce (fresh–cut) not specified/ US	1,358
	Spring mix/ US	1,142
	Spinach (baby)/ US	864
	Spring mix/ US	742
	Spring mix/ US	624
	Spring Mix/US	308
	Leafy Lettuce, Romaine hearts (whole)/ US	296
	Leafy Lettuce, Romaine hearts (whole)/ US	180
	Italian blend (salad blend romaine lettuce)/ US	164
	Baby spinach/ US	162
	Salad blend (iceberg & romaine lettuce base)/ US	120
	Baby spinach/ US	32
	Caesar salad (romaine lettuce base)/ US	30
	Salad blend (iceberg & romaine lettuce base)/ US	30
	Iceberg lettuce (whole)/ US	26
	Field greens (iceberg & romaine lettuce base)/ US	26
	Sprint mix/ US	12
Domestic	Arugula/ Canada	688
	Lettuce mix/ Canada	128
	Baby spinach/ Canada	100
	Spring mix/ Canada	6

Detection of NoV in leafy vegetable samples was observed in most product types. No noticeable differences were identified between product types (Table 7).

Table 7 NoV Positive Rates by Product Type

Product Type	Number of Positive Samples	Number of samples collected	Positive rate (%)
Arugula	1	50	2.0
Lettuce whole (subtotal)	3	132	2.2
Lettuce fresh-cut (subtotal)	10	329	3.0
Baby Spinach	5	371	1.3
Spring mix	6	210	2.9
Swiss Chard	0	11	0
Others (product type not specified)	0	9	0
Total	**25**	**1112**	**2.2**

These results are an indication that contamination of green onions and leafy vegetables with NoV does occur. It only takes a few (1-10) active particles of NoV to cause gastro-enteritis (14). At this time, no studies have been reported to demonstrate how many viral particles, as detected by current PCR-based methodologies, are likely to cause illness (17). Therefore, it is a challenge, even with quantitative results, to determine whether samples positive for NoV represent an actual food safety risk.

4 Conclusion and Discussion

In this targeted survey (2010/11), HAV was not detected in any of the samples, and NoV was not detected in the majority of the samples. NoV was detected and confirmed in three samples of green onions (out of 549) and 25 samples of leafy vegetables (out of 1112). Positive results indicate that the products came in contact with the virus at some point of the production and distribution chain, suggesting that GAPs or GMPs were not followed or appropriately implemented. Immediate follow-up activities were not possible as the types of products examined during this survey had a very short shelf-life and were no longer on the market by the time the results were confirmed. No NoV or HAV outbreaks associated with the consumption of these products were reported during this survey. Based on laboratory results alone, it is not possible to determine whether the positive samples were capable of causing illness.

While the international scientific community is striving to harmonize analytical methods, establish assessment criteria and define prevention and mitigation strategies for viruses in foods, the CFIA is gathering evidence on the prevalence of pathogenic viruses in priority food products through targeted surveys. This work contributes to increase the knowledge needed in this emerging field and may help mitigate potential safety issues related to pathogenic viruses in produce.

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. Surveillance activities will continue and the CFIA will inform stakeholders of its findings.

5 Acknowledgment

We would like to express our sincere thanks to Judy D. Greig, Laboratory for Foodborne Zoonoses, Public Health Agency Canada, for providing the summary of outbreaks (Appendix C).

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Appendix A: List of Acronyms

CDC: Centres for Disease Control and Prevention

CFIA: Canadian Food Inspection Agency

FAO: Food and Agriculture Organization of the United Nations

FDA: *Food and Drugs Act*

FDR: *Food and Drug Regulations*

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

HAV: Hepatitis A virus

HC: Health Canada

NoV: Norovirus

PCR: Polymerase Chain Reaction

PHAC: Public Health Agency of Canada

RT-PCR: Reverse-transcriptase Polymerase Chain Reaction

RT-qPCR: Reverse-transcriptase and Real-time Polymerase Chain Reaction

USFDA: United States Food and Drug Administration

WHO: World Health Organization

g: gram

Appendix B: Summary of Global Foodborne Disease Outbreaks Associated With Leafy Green Vegetables (1998 – 2010) *

Type of Pathogens	Number of Outbreaks	Percentage of Outbreaks
Norovirus	27	29.0
Hepatitis A virus	2	2.2
Other virus pathogen	1	1.1
Subtotal –Viruses	***30***	***32.3***
E. coli O157	27	29.0
Other *E. coli*	5	5.4
Salmonella	19	20.4
Shigella	3	3.2
Campylobacter	3	3.2
Clostridium perfringens	2	2.2
Yersinia	1	1.1
Subtotal –Bacteria	***60***	***64.5***
Cryptosporidium	1	1.1
Cyclospora	2	2.2
Subtotal- Parasites	***3***	***3.2***
Total	**93**	**100**

Summarized according to the information 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: Global Foodborne Disease Outbreaks Associated With Leafy Green Vegetables Contaminated with Viral Pathogens (1998-2010)*

Case #	Year	Month	Microorganism	Vehicle	Country	Province/ State	Number of Cases	Source of Information
1	1999	August	Norovirus	Lettuce	USA	Minnesota	27	Minnesota Dept of Health 1999
2	1999	October	Norovirus	Lettuce	USA	West Virginia	16	CDC
3	2001	April	Norovirus	Lettuce	USA	Maine	70	CDC
4	2002	June	Norovirus	Lettuce	USA	Ohio	15	CDC
5	2002	December	Norovirus	Lettuce	USA	Minnesota	4	CDC
6	2003	March	Norovirus	Lettuce	USA	Minnesota	45	CDC
7	2003	September	Norovirus	Lettuce, romaine	USA	Florida	52	CDC
8	2004		*Other Viral*	Lettuce	Finland	N/A	150	European Food Safety Authority
9	2004	February	Norovirus	Lettuce	USA	Connecticut	13	CDC
10	2004	June	Norovirus	Lettuce	USA	Colorado	15	CDC
11	2004	October	Novorius	Lettuce	USA	Minnesota	9	Minnesota Dept Health 2004
12	*2004*	*December*	*Novorius*	*Lettuce*	*USA*	*Arizona*	*38*	*CDC*
13	2005	February	Norovirus	Lettuce	USA	Minnesota	30	Minnesota Dept. Health 2005
14	2005	September	Hepatitis A (HAV)	Lettuce	USA	California	60	LA Times
15	2006	April	Norovirus	Lettuce	USA	Caluifornia	3	CDC
16	2006	May	Norovirus	Lettuce	USA	Indiana	24	CDC
17	2007	January	Norovirus	Lettuce	USA	Indiana	9	CDC
18	2007	February	Norovirus	Lettuce	USA	Tennessee	8	CDC
19	2007	June	Norovirus	Lettuce	USA	Washington	128	CDC
20	2008		Hepatitis A (HAV)	Lettuce, romaine	USA	California	22	CDC
21	2008		Norovirus GII	Lettuce wraps	USA	Oregon	151	CDC
22	2008		Norovirus	Lettuce based salads	USA	Connecticut	30	CDC

Case #	Year	Month	Microorganism	Vehicle	Country	Province/ State	Number of Cases	Source of Information
23	2008		Norovirus GII	Lettuce based salads	USA	Oregon	19	CDC
24	2008		Norovirus GII	Lettuce based salads	USA	Ohio	11	CDC
25	2009		Norovirus	Lettuce	USA	New York	24	CDC
26	2009		Norovirus GII	Lettuce, leaf	USA	Wisconsin	16	CDC
27	2010	April	Norovirus	Baby mixed greens	USA	Minnesota	35	Minnesota Department of Health
28	2010		Norovirus	Lettuce	Denmark	N/A	260	Eurosurveillance, 2010 15:6
29	2010		Norovirus	Lettuce, romaine	Norway	N/A	157	European line list 2010
30	2010		Norovirus	Lettuce	Denmark	N/A	14	EU 2010 Report

* 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 D Summary of Global Foodborne Disease Outbreaks Associated With Green Onions (1996 – 2010) *

Case #	**Year**	**Month**	**Microorganism**	**Vehicle**	Country	Province/ State	**Number of Cases**	**Source**
1	1996		Hepatitis A Virus*	Green onions	USA	California	60	CDC line list 1996
2	1997		Cryptosporidium parvum	Green onions (suspected)	USA		54	US FDA: Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce, Chapter IV
3	1998		Hepatitis A Virus	Green onions	USA	Ohio	43	J Infect Dis 2001 18398):1273-6
4	2000		Hepatitis A Virus	Green onions	USA	multistate	32	Outbreak alert database, Center for Science in the Public Interest
5	2003		Hepatitis A Virus	Green onions	USA		742	MMWR November 28, 2003. 52(47);1155-1157
6	2006		*Escherichia coli* O157:H7	Green Onions (suspected)	USA	Pennsylvania, Delaware, South Carolina and Utah.	300	CDC
7	2007		Norovirus	Green onions	USA		13	CDC line list 2007
8	2010		*Salmonella* Oranienburg	Green onions	Canada	Ontario	25	CFIA

* 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 E: Analytical Methods Used for Microbial Analysis

Microbial Analysis	Method Identification Number	Title of Method
Hepatitis A Virus	CFIA-VAD-03 (internal, modified version of OLFP-03*)	Method of concentration and purification of virus in clinical food of interest using magnetic beads oligo (dT) 25
	CFIA-VAD-04 (internal, modified version of OLFP-07*)	Detection of HAV using conventional RT-PCR
Norovirus (GI and GII)	CFIA-VAD-03 (internal, modified version of OLFP-03*)	Method of concentration and purification of virus in clinical food of interest using magnetic beads oligo (dT) 25
	CFIA -VAD-06 (internal, modified version of OLFP-10*)	Detection of Norovirus GI using conventional RT-PCR
	CFIA -VAD-07 (internal, modified version of OLFP-10*)	Detection of Norovirus GI using real time RT-PCR
	CFIA -VAD-12 (internal, modified version of OLFP-10*)	Detection of Norovirus GII using conventional RT-PCR
	CFIA-VAD-11 (internal, modified version of OLFP-10*)	Method for cloning, sequencing and molecular characterization of viral genomic fragments amplified by molecular methods

**Compendium of Analytical Methods* (15)..

CFIA-VAD methods have been validated for all commodities analysed. Modifications to the OPFLP methods as published in the Health Canada Compendium site are as follows:

Murine norovirus (MNV-1) was incorporated as a positive control in the elution and extraction protocols. Additionally, samples analysed by CFIA-VAD methods exhibiting a Ct value with the NoV primers and probe set (when the No Template Control reactions are negative) were considered presumptive positive. The technique used for confirmation of the amplified fragments by cloning and sequencing described in section 11 of OPFLP-10, "Preparation of the cDNA clone for Real-Time RT-PCR Standard Curve" was performed on fragments from all presumptive positive samples. For the standard curve, an RNA transcript is used rather than a plasmid control. Automated methods (Qiacube, QIAxcel) were used for DNA purification/extraction and verification of amplification product, respectively. Additionally, the Qiagen Minelute gel extraction kit was used in place of the Qiagen QIAquick method indicated in OPFLP-10. For the RT-qPCR positive amplication controls, CFIA-VAD methods use a segment of transcribed RNA rather than using purified NoV GI and/or GII RNA that was previously

confirmed as positive in other experiments or corresponding cDNA clone, as the use of a segment of transcribed RNA acts as a control for the Reverse-Transcriptase step as well. Lastly, the standard curve for real-time RT-PCR systems used in CFIA-VAD methods is generated using a serially diluted RNA transcript of known concentration.