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

Food Safety Action Plan

REPORT

2011/2012 & 2012/2013 Targeted Surveys

Targeted Surveys Investigating Viral Pathogens and Generic *E. coli* in Fresh Produce

RDIMS#6014459

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. The viruses most frequently implicated in foodborne illnesses are norovirus (NoV) and hepatitis A virus (HAV) but other viruses such as human rotavirus (HRV) have also been found to be transmitted by food. 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. Numerous outbreaks associated with viral contamination of fresh fruits and vegetables have been reported worldwide over the last decade. Fresh produce can become contaminated with 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 fruits and vegetables for extended periods of time, and may cause illness if ingested.

Considering the factors mentioned above and their relevance to Canadians, a variety of fresh produce has 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 2011/12 and 2012/13 targeted surveys was to generate baseline surveillance data on viral pathogens NoV, HAV and Rotavirus, as well as on generic *E. coli*, a bacterial indicator of fecal contamination (tested in 2012/13 only), for imported and domestically produced fresh fruits and vegetables available in the Canadian market. In total, 3,339 samples of pre-packaged fresh fruits and vegetables, including imported and domestically produced bell peppers, broccoli, cabbage, organic tomatoes, lettuces, green onions, fresh-cut leafy and non-leafy vegetables and berries, were collected and analyzed. Levels of generic *E. coli* were found to be acceptable in the 1,959 samples analyzed for this indicator bacteria. HAV was not detected in any of the samples tested, while NoV was detected in 34 samples and HRV was detected in one sample. 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, HRV or HAV outbreaks associated with the consumption of these products were reported in Canada during this survey. As current methods for virus detection are molecular-based assays that do not differentiate 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 Canadian Food Inspection Agency 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.

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 are collected over several 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 Code 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 79-2012)[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 Fruits and Vegetables

2.1 Rationale

In recent years, viruses have been increasingly recognized as a major cause of foodborne illnesses. Outbreak information provided by the Public Health Agency of Canada (PHAC) indicates that produce contaminated with human enteric viruses (i.e., viruses that can multiply in the gastrointestinal tract of humans) have been responsible for at least 140 outbreaks worldwide over the last decade[11] (see examples in Appendix B). The majority of these reported outbreaks were caused by norovirus (NoV), but other viruses such as hepatitis A virus (HAV) and human rotavirus (HRV) have also been implicated in several outbreaks.

Produce can become contaminated with viruses pathogenic to humans during production, harvest, post-harvest handling, processing, packaging, and distribution[12]. The main source of food contamination with enteric viruses is feces and vomit from infected individuals. Therefore, produce can become contaminated in the field by the use of irrigation water contaminated by human sewage. Many fresh fruits and vegetables require extensive handling during harvesting and packaging, and can therefore become contaminated by infected handlers. During processing, the use of contaminated water for produce rinsing, cooling and icing also represents a potential source of virus introduction. 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 identified NoV, HAV and HRV as foodborne viruses of main concern, and determined that NoV and HAV in fresh produce was one of 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)[12].

Based on the above information and the Food Safety Science Committee's recommendations[3], fresh fruits and vegetables 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 a variety of fresh fruits and vegetables available to Canadians at retail.

2.2 Targeted Microorganisms of Concern

2.2.1 Viral Pathogens

Norovirus (NoV), hepatitis A virus (HAV), and human rotavirus (HRV) are enteric viruses that can be transmitted through contaminated food and cause illness.

NoV is considered to be the leading cause of domestically acquired foodborne illnesses in the U.S.[13] and Canada[14]. Generally, NoV causes acute gastroenteritis without long term effects, but can lead to severe dehydration and hospitalization in certain cases. There are currently five recognized NoV genogroups (GI to GV); Genotypes I and II are known to be responsible for most human illnesses[15].

Rotavirus is the leading cause of severe gastroenteritis in young children worldwide, and can also cause persistent diarrhea in immuno-compromised individuals[16]. It has been estimated that only 1% of HRV cases are foodborne[17] (as opposed to person to person transmission). Three serological groups (Type A, B and C) have been identified as pathogenic to humans.

Although the incidence of HAV foodborne illness is much lower than NoV and HRV[14], HAV infection can cause severe symptoms and/or outcomes. 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)[15]. Contamination with HAV is particularly of concern in most developing countries where infection with this virus is endemic[12].

Unlike bacterial pathogens, viral pathogens do not multiply in food since they need to enter living cells to replicate[12]. They are however more environmentally resistant than many bacteria and can remain viable in foods for a very long time[12]. Vegetables (e.g., leafy greens, herbs, and green onions), fruits (e.g., berries), RTE-foods (e.g., salad, sandwiches) and shellfish (e.g., oysters) have been implicated in NoV and HAV associated foodborne outbreaks[12].

2.2.1 Generic *E. coli* - an Indicator of Fecal Contamination

Typically, *E. coli* bacteria that inhabit the large intestines of humans and animals are harmless. Due to their regular presence in the stools of humans and animals, the occurrence of *E. coli* in foods indicates direct or indirect contamination with fecal matter[18]. The presence of generic *E. coli* in foods can also indicate potential contamination with pathogenic enteric microorganisms 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 microorganisms

but does not conclusively indicate that these pathogens 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 production and the time of sale.

2.3 Sample Collection

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 2011 and March 2013.

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 150 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[19]. 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

A total of 3,339 samples of pre-packaged fresh produce were collected and analyzed for NoV, HRV and HAV (Table 1). Samples collected in 2012-13 (n=1,959) were also analyzed for generic *E. coli,* an indicator of fecal contamination.

Table 1 Sample Distribution by Product Type and Origin

Produce group	Imported	Domestic	Total
Bell Pepper[1]	1	144	145
Cabbage[1]	115	8	123
Broccoli[1]	135	3	138
Lettuces[1]	106	31	137
Tomatoes[2] (all organic)	218	189	407
Fresh-Cut Leafy Vegetables[2] (e.g., salad mixes, fresh-cut lettuce, spinach, spring mixes)	252	71	323
Green Onions[3]	496	349	845
Fresh-Cut Vegetables (e.g., slaws, florets, carrots, celery, mushrooms, bell peppers, etc. mixed or not)	332	228	560
Berries[4]	376	285	661
Total	2,031	1,308	3,339

1. Only sampled and tested in 2011-12 (whole products only).
2. Only sampled and tested in 2012-13.
3. 25% of the green onions collected were sold as organic – these organic samples were mostly collected in 2011-12
4. Berries consisted of fresh blueberries (283 samples), strawberries (198 samples), blackberries (108 samples), raspberries (63 samples), cranberries (5 samples), and unspecified berries (4 samples).

All domestic samples (Table 1) were grown and collected in various provinces across Canada. Most imported produce sampled originated from the US (more than 93% of the samples under each product type), except for imported green onions and tomatoes that predominantly originated from Mexico (82% and 76% respectively) and imported berries that predominantly originated from Latin America (63% between Mexico, Chile, Argentina and Guatemala).

2.5 Methods Details

The samples were analyzed for HAV, NoV (GI and GII) and HRV using modified versions of methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*[20] (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 for NoV and HAV 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.

For the analysis of generic *E. coli* (done in fiscal year 2012/13 only), the MFHPB 27 method published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*[20] (Appendix C) was used. This method is used for regulatory testing by the CFIA and is fully validated for the analysis of fresh fruits and vegetables. Based on the interpretation of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*[21], enumeration results were reported as follow: acceptable if levels were below 100 CFU/g, marginal if levels were between 100 and 1,000 CFU/g, and unacceptable if levels were above 1,000 CFU/g,

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 of human enteric viruses NoV, HRV 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 three common viral pathogens (i.e., NoV, HRV 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

3.1 Virus Results

A total of 3,339 samples were analyzed for HAV, HRV and NoV. HAV was not detected in any of the samples tested. HRV was detected in only one sample of berries. NoV was detected in 34 samples (1.02%), in all produce groups sampled except bell peppers and tomatoes (Table 2).

Table 2. Summary of Virology Results

Produce Group	Number of Samples	NoV (GI and GII)		HAV		HRV	
		Detected in 25g	Not Detected in 25g	Detected in 25g	Not Detected in 25g	Detected in 25g	Not Detected in 25g
Bell Pepper	145	0	145	0	145	0	145
Cabbage	123	3 (2.44%)	120	0	123	0	123
Broccoli	138	2 (1.45%)	136	0	136	0	136
Lettuces	137	1 (0.73%)	137	0	137	0	137
Tomatoes (all organic)	407	0	407	0	407	0	407
Fresh-Cut Leafy vegetables	323	3 (0.93%)	317	0	323	0	323
Green Onions	845	11 (1.30%)	834	0	845	0	845
Fresh-Cut Vegetables	560	4 (0.71%)	558	0	560	0	560
Berries	661	10 (1.51%)	651	0	661	1 (0.15%)	660
Total	**3339**	**34 (1.02%)**	**3305 (98.98 %)**	**0**	**3339**	**1 (0.03%)**	**3338 (99.97 %)**

Positive samples originated from the USA, Mexico and Canada. Both genotypes GI and GII were identified in the NoV positive samples. Type A HRV was identified in one sample of raspberry (Table 3).

Table 3 Summary of Positive Results for Norovirus (GI and GII) and Rotavirus detected in Fresh Fruit and Vegetable Samples

Product Type/ Country of Origin	Virus Type
Cabbage:	
1 sample from Mexico	NoV GII
2 samples from the U.S.A.	NoV GI (1 sample) and NoV GII (1)
Broccoli:	
1 sample from the U.S.A. (organic)	NoV GI
1 sample from the U.S.A.	NoV GII
Lettuce:	
1 sample of iceberg lettuce from the U.S.A.	NoV GI
Fresh-Cut Leafy Vegetables:	
1 sample of salad mix from Mexico	NoV GI
2 samples of salad mix from the U.S.A.	NoV GI (1 sample) and NoV GII (1)
Green Onions:	
7 samples from Mexico (organic)	NoV GI (5 samples) and NoV GII (2)
2 samples from the U.S.A. (organic)	NoV GI
1 sample from Canada	NoV GI
1 sample from Canada (organic)	NoV GI
Fresh-Cut Vegetables:	
1 sample of mixed vegetables from Canada	NoV GII
3 samples of coleslaw from the U.S.A.	NoV GI (1 sample) and NoV GII (2)
Berries:	
3 samples of strawberry from the U.S.A.	NoV GI
1 sample of strawberry from Canada	NoV GI
1 sample of blueberry from the U.S.A.	NoV GI
2 samples of blueberry from Canada	NoV GI
1 sample of blackberry from Mexico	NoV GI
1 sample of blackberry from Canada	NoV GI
1 sample of raspberry from Mexico	NoV GI
1 sample of raspberry from Mexico	HRV type A

Real-Time RT-PCR (RT-qPCR) was performed to try and estimate the number of virus genomic copies in the samples that tested positive for NoV (the assay was not available for quantification of HRV at the time of this study). The estimated number of virus copies was obtained for 12 out of the 34 NoV positive samples, and ranged from to 7 to 346 genomic copies per 25 grams of product. However, the poor sensitivity of the current methods, mainly due to several challenges associated with the extraction of viruses from foods, must be kept in mind when considering these results. Moreover, it only takes a few (1-10) active particles of viruses to cause gastro-enteritis[15]. 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[22]. Therefore, it is a challenge, even with quantitative results, to determine whether positive samples represent an actual food safety risk.

The above results (Tables 2 and 3) are an indication that contamination of fresh fruits and vegetables with NoV and HRV does occur sporadically.

3.2 Generic *E. coli* Results

All samples collected in 2012/13 (n=1,959) were additionally analyzed for generic *E. coli*. Levels of generic *E. coli* were found to be acceptable in all the samples (Table 4).

Table 4. Summary of Results for Generic *E. coli* Analysis

Produce Group	Number of Samples	Generic *E. coli* Levels		
		<100 CFU/g (acceptable)	100-1,000 CFU/g (marginal)	>1,000 CFU/g (unacceptable)
Tomatoes (all organic)	407	407	0	0
Fresh-Cut Vegetables	430	430	0	0
Green Onions	409	409	0	0
Fresh-Cut leafy vegetables	323	323	0	0
Berries	390	390	0	0
Total	**1,959**	**1,959**	**0**	**0**

Note that of these 1,959 samples, 10 were positive for NoV or HRV. Generic *E. coli* are a group of bacteria used as an indicator of fecal contamination from either human or animal sources. Their presence at higher levels in a sample is an indication that GAPs or

GMPs were not followed at some point during production or distribution. Enteric viruses such as NoV, HAV and HRV originate from human sources only, therefore their presence in food is an indication that contamination of human origin, via sewage or infected workers, occurred. Viruses are environmentally more resistant than bacteria and may persist for longer periods of time in the environment; therefore they can be present even in the absence of fecal indicators[6, 17].

4 Discussion and Conclusion

In these targeted surveys (2011/12 and 2012/13), HAV was not detected in any of the samples, while HRV and NoV were detected on one and 34 samples respectively. 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, HRV or HAV outbreaks associated with the consumption of these products in Canada 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.

Levels of generic *E. coli* were found to be acceptable in all the samples analysed for this indicator bacteria in 2012/13. Generic *E. coli* is typically used as an indicator of fecal contamination to obtain clues on whether GAPs or GMPs were followed along the production and distribution chain. However, the detection of virus genomic material in a sample is also an indication that fecal contamination of human origin occurred before the point of sale. Therefore, the positive results obtained during this survey suggest that GAPs or GMPs were not followed or appropriately implemented for some of the products analyzed.

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 Acknowledgement

We would like to express our sincere thanks to Judy D. Greig, Public Health Agency of Canada, for providing outbreak information (Appendix B).

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Appendix A: List of Acronyms

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

HRV: Human rotavirus

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

WHO: World Health Organization

g: gram

Appendix B: Examples of Major Outbreaks (>100 cases) Associated with Fruits and Vegetables Contaminated with Viral Pathogens (2004-2013)*

Year	Microorganism	Vehicle	Country	Number of Cases	Narrative
2004	Norovirus	Melon	United States	100	
2005	Norovirus	Raspberries	Denmark	1000	Imported to Denmark from Poland in spring 2005.
2007	Norovirus	Lettuce	United States	128	
2008	Norovirus, GII	Lettuce	United States	151	lettuce wraps; 1 hospitalized
2009	Norovirus	Raspberries	Finland	121	Imported from Poland. Restaurant. frozen raspberries
2009	Norovirus	Raspberries	Sweden	130	School, kindergarten
2009	Norovirus	Salad	Germany	101	Military base - retrospective cohort study Of 27 cases (AR 15.2%), 25 had eaten at the canteen and 21 had consumed salad.
2009	Norovirus, GII	Salad	United States	131	2 hospitalized
2009	Norovirus	Salad	Germany	102	Salads offered as buffet; Canteen or workplace catering
2009	Norovirus	Raspberries	Finland	130	School, kindergarten
2009	Hepatitis A virus (HAV)	Tomatoes	Australia	155	Appears to be linked to semi-dry tomatoes.
2009	Norovirus, GII	Raspberries	Finland	128	Imported from Poland. Restaurant. GI.4; frozen raspberries
2009	Norovirus, GII	Raspberries	Finland	525	Imported from Poland. More than 500 cases, GII.b Hilversum/1999; frozen raspberries (mixed in curd cheese as a snack); kindergarten
2009	Hepatitis A virus (HAV)	Tomatoes	Australia	200	On-going outbreak of HAV in Australia that has sickened about 200 people and appears to be linked to semi-dry tomatoes.

Year	Microorganism	Vehicle	Country	Number of Cases	Narrative
2010	Norovirus	Lettuce	Norway	157	Lollo lettuce
2010	Rotavirus	Fruit - bananas apples citrus fruits	Russia	200	200 children hospitalised after eating fruits imported from China
2011	Norovirus, GI	Uncooked vegetables	France	147	Cases among those parachuting at night and some physicians. Ill cook positive for norovirus by PCR - same genogroup I norovirus found in carrots, salad and tomatoes served at dinner.
2012	Norovirus	Strawberries	Germany	11200	32 hospitalized. Largest wave of foodborne illness recorded in Germany. Wholesaler sold contaminated frozen strawberries to commercial kitchens of 3 companies that made cafeteria food for schools and kindergartens. Strawberries likely from China.
2013	Hepatitis A virus (HAV)	Pommegranate Seeds	United States	162	"On June 4, 2013, Townsend Farms, Inc. of Fairview, Oregon voluntarily recalled certain lots of its frozen Organic Antioxidant Blend because of potential hepatitis A virus contamination.

* 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

Microbial Analysis	Method Identification Number	Title of Method
Hepatitis A Virus	CFIA-VAD-02 (CFIA method)	Method to Concentrate and Purify Viruses of Clinical Interest from Food Using Magnetic Cationic Beads.
	CFIA-VAD-03 (internal, modified version of OPFLP-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 OPFLP-07*)	Detection of HAV using conventional RT-PCR
Norovirus (GI and GII)	CFIA-VAD-02 (CFIA method)	Method to Concentrate and Purify Viruses of Clinical Interest from Food Using Magnetic Cationic Beads.
	CFIA-VAD-03 (internal, modified version of OPFLP-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 OPFLP-10*)	Detection of Norovirus GI using conventional RT-PCR
	CFIA -VAD-07 (internal, modified version of OPFLP-10*)	Detection of Norovirus GI using real time RT-PCR
	CFIA -VAD-12 (internal, modified version of OPFLP-10*)	Detection of Norovirus GII using conventional RT-PCR
	CFIA-VAD-11 (internal, modified version of OPLFP-10*)	Method for cloning, sequencing and molecular characterization of viral genomic fragments amplified by molecular methods
Rotavirus	CFIA-VAD-02 (CFIA method)	Method to Concentrate and Purify Viruses of Clinical Interest from Food Using Magnetic Cationic Beads.
	CFIA-VAD-08 (based on OPFLP-04 RV-A RT-PCR section)	Method to detect Rotavirus (RV-A) by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR).

	CFIA-VAD-11 (internal, modified version of OPFLP-10*)	Method for cloning, sequencing and molecular characterization of viral genomic fragments amplified by molecular methods
Generic *E. coli*	MFHPB-27 (September 1997)	Enumeration of Escherichia coli in Foods by the Direct Plating (DP) Method

**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.