Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2011-2012 Targeted Surveys

Targeted Survey Investigating Bacterial Pathogens in Sprout Seeds and in Sprouts

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.

Sprouts have been reported to be responsible for numerous outbreaks of foodborne illness around the world. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has ranked sprouts as a high priority in terms of microbiological hazards among fresh fruits and vegetables. Contamination in sprouts often originates from the seeds. Growers are not always aware that the seeds they produce will be used for human consumption, and may therefore not be as diligent in preventing contamination during production. The sprouting process requires warm and humid conditions that are ideal for the proliferation of bacterial pathogens that could be present on or in the seeds. The presence of pathogens in sprouts creates a potential risk for foodborne illness as sprouts are usually consumed raw or lightly cooked. *Salmonella* and *Escherichia coli* (*E. coli*) O157 have been identified as the primary bacterial pathogens of concern in sprouts and their seeds.

Considering the above factors and their relevance to Canadians, sprouts, including seeds for sprouting, have been selected as one of the priority commodity for enhanced surveillance under the FSAP. Over the course of this baseline study (2011/12 to 2014/15), approximately 4,000 sprout and sprout seed samples will be collected from retail locations and tested for the presence of various pathogens of concern. The main objectives of this targeted survey (2011/12) were to generate baseline surveillance data on the bacterial pathogens *Salmonella* and *E. coli* O157 in sprout seeds as well as the priority verotoxigenic *E. coli* (VTEC) O157, O26, O111, O103 and O145 in sprouts available in the Canadian market. A total of 419 samples of sprout seeds and 264 samples of sprouted seeds (i.e. sprouts) were collected at retail during this survey. *E. coli* O157 was not detected in any of the sprout seed samples while *Salmonella* was detected in one sample (0.2%). The *Salmonella* positive result triggered appropriate follow-up procedures including the recall of the affected product. None of the priority VTECs tested for were detected in the sprout samples analysed during this survey. These results suggest that the sprout seeds and sprouts sampled during this survey were generally produced under Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs).

The Canadian Food Inspection Agency (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 strengthen Canada's 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) Food Safety Action Plan[2] (FSAP) is one element of the government's broader FCSAP initiative. The goal of the 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 will be 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 and are aimed 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.

This targeted survey (2011/12) represents part of the collection of over 4,000 sprout and sprout seed samples over four years (2011/12 to 2014/15) and was designed to gather

baseline information on the occurrence of microbial pathogens of concern in sprouts and sprout seeds available to Canadians at retail.

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 sprouts are encouraged to follow these international codes of practice. Of relevance for this survey are the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CAC/RCP 53-2003), with Annex II on the production of sprouts[3], the *Recommended International Code of Practice-General Principles of Food Hygiene* (CAC/RCP 1-1969)[4], Health Canada's *Policy on Managing Health Risk Associated with the Consumption of Sprouted Seeds and Beans*[5], and the CFIA *Code of Practice for the Hygienic Production of Sprouted Seeds*[6]. These codes address GAPs and GMPs which, when applied, control and reduce the potential for contamination of fresh fruits and vegetables with microbial, chemical, and physical hazards at all stages of the production from primary production to packaging.

Sprouts and sprout seeds 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.

Sprouts that are imported or domestically produced and marketed inter-provincially must also comply with the 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 labelled.

The *Fresh Fruit and Vegetable Regulations,* and the food-related sections of the FDA and FDR are enforced by the CFIA.

The FSAP 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 Sprout Seeds and Sprouts

2.1 Rationale

Sprouts have been reported to be responsible for numerous outbreaks of foodborne illness worldwide. From 1996 to 2012, there were 72 documented outbreaks associated with sprouts contaminated with microbial pathogens (Appendix B). These outbreaks were mostly associated with the bacterial pathogen *Salmonella.* A few outbreaks were also associated with pathogenic *Escherichia coli (E. coli*) O157 and other VTECs.

In most sprout related outbreaks, the seeds used for sprouting were identified as the source of contamination[11, 12]. Since sprout seeds are mostly produced for agricultural purposes, growers may not know beforehand that the seeds will be used for human consumption and therefore may not implement GAPs to prevent contamination[12, 13]. Seeds can become contaminated with bacterial pathogens in the field by the use of untreated manure or irrigation water, and by feces of grazing animals. The seeds can also be contaminated by unclean equipment and conditions during harvest, storage, transportation and handling. Sprouts are a factory crop grown in warm and humid conditions that are ideal for bacterial proliferation[11]. Any bacterial pathogen present on or inside the seeds can therefore grow exponentially during the sprouting process. Decontamination of seeds before sprouting is strongly recommended[7, 11] but is not always done and even when done may not completely eliminate certain pathogens when present. Contamination of sprouts may also occur during production, transportation and storage of the final product if adequate sanitation and hygiene practices are not followed[11]. The presence of pathogens in sprouts creates a potential risk for foodborne illness as sprouts are usually consumed raw or lightly cooked.

Sprouts were classified in the second highest priority group of concern in terms of microbiological hazards among fresh fruits and vegetables during a joint FAO/WHO Expert Meeting in 2007[14]. This was based on multiple factors such as historical outbreaks and potential for contamination by pathogens.

Based on the above information and the Food Safety Science Committee's recommendations, sprout seeds and sprouts have been selected for enhanced surveillance under the FSAP. The overall objective of this surveillance is to gather baseline information on the occurrence of pathogens of concern in sprout seeds and sprouts available to Canadians at retail.

This targeted survey (2011/12) is part of the information collection, with a focus on investigating the presence and distribution of bacterial pathogens (*E. coli* O157:H7/NM and *Salmonella*) in sprout seeds, as well as the presence and distribution of priority serogroups of non-O157 VTEC in sprouts, available on the Canadian market.

2.2 Targeted Microorganisms – *Salmonella*, *E. coli* O157 and other Verotoxigenic *E. coli*

Salmonella normally lives in the intestines of animals such as poultry, swine, wild birds, domestic pets and reptiles[15]. Therefore, *Salmonella* contamination often occurs in food of animal origin (e.g., poultry, eggs and milk). However, cases of salmonellosis have been increasingly reported to be associated with the consumption of contaminated fruits and vegetables[16].

A few strains of *E. coli* are capable of causing human disease. For example, the Verotoxigenic *E. coli* (VTEC) strains can produce Shiga-like toxins and cause severe diarrhea. This class of *E. coli* includes the predominant disease-causing *E. coli* O157:H7/NM[15] and other emerging disease-causing non-O157 *E. coli* (e.g., O26, O103, O111, and O145)[17, 18]. VTECs are found naturally in the intestines of ruminant animals such as cattle, sheep and deer, as well as other animals such as rabbits and pigs. While ground beef is still the most common food source of *E. coli* O157 associated foodborne illnesses[19], fresh produce has also recently emerged as a significant source of *E. coli* O157 and other VTEC related illnesses[16, 17].

Sprout seeds (and therefore sprouts) can become contaminated with microbial pathogens such as *Salmonella* and VTECs in the field by exposure to improperly composted manure, contaminated water, wildlife feces and unclean equipment as well as due to poor hygienic practices of farm workers[11, 13].

2.3 Sample Collection

All samples were collected from national retail chains and local/regional grocery stores as well as other conventional retail and natural food stores located in various cities across Canada. The number of samples collected in various regions across Canada was based on the relative proportion of the population in the respective regions. Samples were collected between April 2011 and March 2012. The samples included both imported and domestic products as well as organic and conventional products.

For 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[20]. Collected samples were required to be shipped under conditions that limit the growth of micro-organisms during transit. Samples were declared "unfit" for analysis if there were issues regarding the conditions in which they were handled or shipped.

2.4 Sample Distribution

2.4.1 Sprout Seed Samples

Table 1. Distribution of Sprout Seed Samples by Product Type

Seed Type	Total
Alfalfa	46 (11.0%)
Amaranth	1 (0.2%)
Arugula	6 (1.4%)
Bean - Adzuki	8 (1.9%)
Bean - Mix	4 (1.0%)
Bean - Mung	87 (20.8)
Broccoli	18 (4.3%)
Buckwheat	8 (1.9%)
Cabbage	1 (0.2)
Canola	1 (0.2%)
Chia	18 (4.3%)
Chick pea	7 (1.7%)
Clover	20 (4.8%)
Cress	4 (1.0%)
Fennel	2 (0.5%)
Fenugreek	7 (1.7%)
Flax	36 (8.6%)
Kale	5 (1.2%)
Kamut	1 (0.2%)
Lentil	1 (0.2%)
Mix	27 (6.4%)
Mustard	15 (3.6%)
Oat	3 (0.7%)
Onion	3 (0.7%)
Pea	2 (0.5%)
Quinoa	1 (0.2%)
Radish	43 (10.3%)
Salba	5 (1.2%)
Sesame	1 (0.2%)
Sunflower	25 (6.0%)
Wheat	7 (1.7%)
Unknown	6 (1.4%)
Total	**419 (100%)**

Table 2. Distribution of Sprout Seed Samples by Country of Origin

Seed Type	Total
Australia	3 (0.7%)
Bolivia	2 (0.5%)
Canada	89 (21.2%)
China	63 (15.0%)
France	1 (0.2%)
Germany	10 (2.4%)
Hungary	9 (2.1%)
India	8 (1.9%)
Italy	17 (4.1%)
Mexico	1 (0.2%)
Myanmar	1 (0.2%)
Peru	6 (1.4%)
Taiwan	3 (0.7%)
Thailand	7 (1.7%)
Turkey	6 (1.4%)
United Kingdom	1 (0.2%)
United States	69 (16.5%)
Imported – Unknown Country	123 (29.4%)
Total	**419 (100%)**

A wide variety of sprout seeds were collected during this survey. About 80% of the seeds collected were sold as organic. The dominant types of seeds collected were mung bean seeds (20.8%), alfalfa seeds (11.0%) and radish seeds (10.3%). While a significant

proportion of seeds were from Canada (21.2%), a large proportion of imported samples (29.4%) were of unknown origin. Note that current Canadian labelling requirements for many pre-packaged products such as seeds do not require companies to label where their products are imported from.

2.4.2. Sprout samples

Table 3. Distribution of Sprout Samples by Product Type

Sprout Type	Number of samples
Alfalfa	76 (28.8%)
Asparagus	1 (0.4%)
Bean sprout:	
- Mung bean	77 (29.2%)
- Soy bean	5 (1.9%)
- not specified	8 (3.0%)
Broccoli	28 (10.6%)
Cabbage	1 (0.4%)
Clover	4 (1.5%)
Fenugreek	4 (1.5%)
Mix	11 (4.2%)
Onion	7 (2.7%)
Pea	16 (6.1%)
Radish	7 (2.7%)
Sunflower	11 (4.2%)
Unknown	8 (3.0%)
Total	264

Over 70% of the samples consisted of bean sprouts, alfalfa sprouts and broccoli sprouts. About 16.7% of the products sampled were labelled as organic. Of the 264 samples of sprouts tested, 262 samples were from Canada (99.2%) and 2 samples were from the United Sates (0.8%).

2.5 Method Details

For the analysis of *Salmonella* and *E. coli* O157, the samples were analyzed using the analytical methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*[21] (Appendix C). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of fresh sprouts and sprout seeds.

For the detection of *Salmonella* and *E. coli* O157, samples were analyzed by cultural presence/absence methods. The laboratories had the option of using rapid Polymerase Chain Reaction (PCR) based screening methods to first screen enrichment broths for the presence of DNA from the pathogen of interest, followed by cultural confirmation of presumptive positives.

If pathogens were detected, the isolates were further characterised by pulsed field gel electrophoresis (PFGE), i.e., DNA fingerprinting, at the CFIA's PFGE Centre. Serotyping for *Salmonella* spp. was performed at the *Salmonella* Typing Laboratory, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada (PHAC) in Guelph, Ontario.

For the detection of VTEC, the method of Gill et al[17] and Blais and Martinez-Perez[18] was used. Briefly, samples were first screened by polymerase chain reaction (PCR)-based methods for the presence of verotoxin genes. Presumptive positive results were confirmed by isolation, purification and identification procedures. For the confirmation of the priority VTEC serotypes (O157, O26, O111, O103 and O145), the probe-based assay CHAS (cloth based hybridization array system) was used[18]. This method targets genes for key virulence factors and determinants specific to the five priority VTEC serotypes.

2.6 Assessment Guidelines

The assessment criteria used in this survey (Tables 4 and 5) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*[22] and associated methods published in Health Canada's *Compendium of Analytical Methods*[21].

Table 4. Assessment Guidelines for Bacterial Pathogens in Sprout Seeds

Bacterial Analysis* (Method Identification Number)	**Assessment Criteria**	
	Satisfactory	**Unsatisfactory**
E. coli* O157:H7/NM* (MFLP-30, Supplement 1 & 2, and MFLP-80)	Absent in 25 g	Present in 25 g
Salmonella* spp.* (MFLP-29 modified and MFHPB-20)	Absent in 25 g	Present in 25 g

* Compendium of Analytical Methods [21].

**No criteria have been established by Health Canada at this time for these bacterial pathogens in sprout seeds. 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 5. Assessment Guidelines for VTEC in Sprouts

Bacterial Analysis (Method Identification Number)	Assessment Criteria	
	Satisfactory	**Unsatisfactory**
Priority VTEC* (serotypes O157, O26, O111, O103 and O145) (Published methods[17, 18])	Absent in 25 g	Present in 25 g

*No criteria have been established by Health Canada at this time for these bacterial pathogens in sprouts. 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.

Samples assessed as unsatisfactory are subject to follow-up actions, such as directed follow-up sampling, inspection of establishment, health risk assessment, and/or product action (e.g., product recall).

2.7 Survey Limitations

Samples tested during this survey were collected at retail locations across Canada, as opposed to monitoring samples that are picked up at distribution points and warehouses. As such, products sampled at retail could be mixed and originate from different shipments and/or suppliers. Though this represents what the Canadian consumer experiences, this imposes certain limitations with respect to the traceability of the products and the identification of the source of contamination in the case of positive results.

Results obtained for a targeted survey sample are from the analysis of a single sample unit. This sampling and testing strategy generally precludes the extrapolation of the laboratory result to the whole production lot as it is not statistically representative. This imposes certain limitations in the interpretation of the results to the specific lot in the absence of additional information.

Finally, given the seasonality, as well as the varying channels of commerce, the source of the products can change dramatically from one season to the next. As such, there is an insufficient number of samples in this survey to carry out a detailed analysis of the results based on a country of origin. In cases of positive results, unsatisfactory rates between countries are not considered to be statistically comparable.

3 Results

A total of 419 sprout seed samples were analysed for *Salmonella* and *E. coli* O157 (Table 6). Bacterial pathogen *E. coli* O157:H7/NM was not detected in any samples. *Salmonella* was detected in one sample of organic sunflower sprouting seeds from Italy. Serotype *Salmonella enterica* Berlin was identified from the positive sample. As a result of this finding, the CFIA initiated a food safety investigation and conducted appropriate follow-up actions, which included the recall of the affected products.

Another 264 samples of sprouts (i.e., sprouted seeds) were also sampled and tested for the five priority serogroups of VTEC (O157, O26, O103, O111, and O145). These serogroups were not detected in any of the samples tested (Table 6).

Table 6: Summary of the Results

Product Type	Number of samples	Assessment	
		Satisfactory	Unsatisfactory
Sprout seeds*	419	418	1
Sprouts**	264	264	0

* Analyzed for *Salmonella* and *E. coli* O157

** Analyzed for VTECs (O157, O26, O103, O111, and O145)

4 Discussion and Conclusion

In this survey (2011/12), *E. coli* O157 was not detected in any of the 419 sprout seed samples tested. However, *Salmonella* was detected in one sample (0.2%). As a result of this finding, the CFIA conducted a food safety investigation and the affected product was recalled. Priority VTECs (O157, O26, O103, O111, and O145) were not detected in any of the 264 samples of sprouts tested. It is important to note that there were no reported illnesses associated with the consumption of any of the products sampled during this survey.

The overall findings of this survey suggest that the sprout seeds and the sprouts sampled during this survey were generally produced and handled under GAPs and GMPs. However, it was found that contamination of commercial sprout seeds with *Salmonella* can occur sporadically and could represent a food safety risk.

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 References

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Appendix A: List of Acronyms and Abbreviations

CFIA: Canadian Food Inspection Agency

CDC: Centres for Disease Control and Prevention

E. coli: *Escherichia coli*

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

MFHPB: Health Protection Branch/ Microbiology Food Health Protection Branch

MFLP: Microbiology Food Laboratory Procedures

NM: non-motile

PCR: Polymerase Chain Reaction

PFGE: Pulsed Field Gel Electrophoresis

PHAC: Public Health Agency of Canada

spp.: species

VTEC: Verotoxigenic *Eschercihia coli*

WHO: World Health Organization

g: gram

Appendix B: Global Foodborne Disease Outbreaks Associated With Sprouts Contaminated with Microbial Pathogens (1996 – March 2012)*

Year	Vehicle	Micro-organisms	Country	Cases	Source
1996	Radish sprouts	*Escherichia coli* O157:H7	Japan	8844	Am J Epidemiol 1999, 150(8):787-96
1996	Alfalfa sprouts	*Salmonella enterica* Montevideo	United States	417	Ann. Intern. Med. 2001, 135:239-247
1996	Alfalfa sprouts	*Salmonella enterica* Stanley	United States	30	CDC line list
1996	Radish sprouts	*Escherichia coli* O157:H7	Japan	47	Rinsho Byori 1997 Sep;45(9):869-74
1997	Alfalfa sprouts	*Salmonella enterica* Senftenberg	United States	60	Ann. Intern. Med. 2001135:239-247
1997	Alfalfa sprouts	*Salmonella enterica* Meleagridis	Canada	78	Food Safety Network
1997	Alfalfa sprouts	*Salmonella enterica* Multiple - Anatum/Infantis	United States	109	Gideon Online
1997	Sprouts	*Salmonella*	United States	60	Gideon Online
1997	Alfalfa sprouts	*Escherichia coli* O157:H7	United States	113	MMWR 1997; 46(32):741-744
1997-98	Alfalfa sprouts	*Salmonella enterica* Senftenberg	United States	54	United States Food and Drug Administration (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

Year	Vehicle	Micro-organisms	Country	Cases	Source
1998	Alfalfa sprouts	*Escherichia coli* O157:H7 - NM	United States	8	Ann. Intern. Med. 2001 135(4):239-247
1998	Alfalfa sprouts	*Salmonella enterica* Cubana	United States	22	Ann. Intern. Med. 2001 135(4):239-247
1998	Alfalfa sprouts	*Salmonella*	United States	18	Gideon Online
1998	Alfalfa sprouts	*Salmonella enterica* Havana	United States	40	Public Health Rep 2000 115(4):339-45
1999	Alfalfa sprouts	*Salmonella enterica* Paratyphi	Canada	51	Can Commun Dis Rep. 2001, 27(16):133-7; discussion 137-8)
1999	Alfalfa sprouts	*Salmonella*	United States	34	CDC line list
1999	Mung bean sprouts	*Salmonella enterica* Enteritidis PT 4	United States	108	CDC line list
1999	Clover sprouts	*Salmonella enterica* Saintpaul	United States	100	CDC line list & Gideon Online
1999	Alfalfa sprouts	*Salmonella enterica* Mbandaka	United States	89	Emerg Infect Dis 2003 9(4):474-479
1999	Alfalfa sprouts	*Salmonella enterica* Typhimurium	United States	119	Food Safety Network
1999	Alfalfa sprouts	*Salmonella enterica* Muenchen	United States	175	J. Clinical Microbiology, 2001, 39(10):3461-3465
2000	Mung bean sprouts	*Salmonella enterica Enteritidis PT 4 11b*	Canada	12	Can Commun Dis Rep. 29(14) 15 July 2003

Year	Vehicle	Micro-organisms	Country	Cases	Source
2000	Mung bean sprouts	*Salmonella enterica Enteritidis*	United States	75	CDC line list
2000	Bean sprouts	*Salmonella enterica Enteritidis*	Netherlands	27	Emerg Infect Dis 2002, 8(4):440-3
2001	Mung bean sprouts	*Salmonella enterica Enteritidis* PT 913	Canada	84	Can Commun Dis Rep. 2001 Sep 15;27(18):151-6.
2001	Mung bean sprouts	*Salmonella enterica* Group D	United States	31	CDC line list & Food and Waterborne Illness Surveillance and Investigation, Annual Report, Florida 2001
2001	Alfalfa sprouts	*Salmonella*	United States	22	Food Safety Network
2001	Alfalfa sprouts	*Salmonella enterica* Kottbus	United States	32	Journal of Food Protection 2003 66(1):1317
2002	Alfalfa sprouts	*Escherichia coli* O157:H7	United States	7	CDC line list
2003	Alfalfa sprouts	*Salmonella enterica* Saintpaul	United States	9	CDC line list
2003	Alfalfa sprouts	*Salmonella enterica* Chester	United States	26	CDC line list
2003	Alfalfa sprouts	*Escherichia coli* O157:H7	United States	6	Epidemiology and Infection (2005) 133:439-447.
2003	Alfalfa sprouts	*Escherichia coli* O157	United States	13	Epidemiology and Infection (2005) 133:439-447.
2003	Alfalfa sprouts	*Salmonella*	United States	6	Food Safety & Security Jan. 2004

Year	Vehicle	Micro-organisms	Country	Cases	Source
2004	Bean sprouts	*Escherichia coli* O157:H7-NM	United States	4	FDA
2004	Alfalfa sprouts	*Salmonella enterica* Bovismorbificans	United States	35	FDA & CDC line list
2004	Alfalfa sprouts	*Salmonella*	United States	12	Food Safety Network
2005	Mung bean sprouts	*Salmonella enterica Enteritidis* PT 13	Canada	247	Kingston Public
2005	Mung bean sprouts	*Salmonella enterica Enteritidis* PT 33	Canada	8	NML Annual Report 2005
2005	Alfalfa sprouts	*Salmonella enterica* Oranienburg	Australia	125	OzFoodNet 2005 Annual Report
2006	Bean sprouts	*Salmonella enterica* Braenderup	United States	4	CDC line list
2006	Bean sprouts	*Salmonella enterica* Saintpaul	Australia	11	OzFoodNet sites, 2006 1 January to 31 March
2006	Mung bean sprouts	*Salmonella enterica* Multiple - Bareilly/Virchow	Sweden	105	The Local - Sweden
2007	Alfalfa sprouts	*Salmonella enterica* Mbandaka	United States	15	CDC line list
2007	Bean sprouts	*Salmonella enterica* Montevideo	United States	24	CDC line list
2007	Mung bean sprouts	*Salmonella*	Sweden	115	Eurosurveillance monthly 2007, 12(11)
2007	Alfalfa sprouts	*Salmonella enterica* Stanley	Sweden	51	Eurosurveillance weekly 18 October 2007

Year	Vehicle	Micro-organisms	Country	Cases	Source
2007	Alfalfa sprouts	*Salmonella enterica* Welteverden	Multiple	45	Eurosurveillance weekly 29 Nov 2007
2008	Sprouts	*Listeria monocytogenes*	United States	20	CDC line list 2008
2008	Alfalfa sprouts	*Salmonella enterica* Typhimurium	United States	24	Marler Clark
2008	Alfalfa sprouts and iceberg lettuce	*Escherichia coli* O157:NM	United States	21	CDC line list 2008
2008	Alfalfa sprouts	*Salmonella enterica* Typhimurium	United States	13	Marler Clark website
2009	Sprouts	*Salmonella enterica* Cubana	United States	2	CDC line list 2009
2009	Alfalfa sprouts	*Salmonella enterica* Oranienburg	United States	25	CDC line list 2009
2009	Alfalfa sprouts	*Salmonella enterica* Typhimurium	United States	14	CDC line list 2009
2009	Alfalfa sprouts	*Salmonella enterica* Saintpaul	United States	256	MMWR 09/01/20
2009	Sprouts	*Salmonella enterica* Cubana	Canada	20	PHAC
2009	Alfalfa sprouts	*Salmonella enterica* Bovismorbificans	Finland	28	The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009
2009	Sprouts	*Salmonella enterica* Bovismorbificans	Estonia	6	The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009

Year	Vehicle	Micro-organisms	Country	Cases	Source
2009	Alfalfa sprouts	*Salmonella enterica* Oranienburg	United States	25	CDC line list
2009	Alfalfa sprouts	*Salmonella enterica* Typhimurium	United States	12	Michigan Department of Community Health
2009	Alfalfa sprouts	*Salmonella enterica* Saintpaul	United States	228	MMWR 09/01/2009
2009	Sprouts	*Salmonella enterica* Cubana	Canada	20	PHAC
2009	Alfalfa sprouts	*Salmonella enterica* Bovismorbificans	Finland	28	The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009
2009	Sprouts	*Salmonella enterica* Bovismorbificans	Estonia	6	The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009
2010	Bean sprouts	*Salmonella enterica* Bareilly	United Kingdom	231	Health Protection Report 2011, 5(10)
2010	Alfalfa sprouts	*Salmonella enterica* Enteritidis	United States	21	CDC
2010	Alfalfa sprouts	*Salmonella enterica* Newport	United States	28	Bites, Kansas State University
2010	Alfalfa sprouts	*Salmonella enterica* I4,[5],12:i:-	United States	125	Illinois Department of Public Health
2010	Clover sprouts	*Salmonella*	United States	7	Sprouters Northwest Inc. of Kent, Wash
2011	Clover sprouts	*Escherichia coli* O26	United States	29	CDC

Year	Vehicle	Micro-organisms	Country	Cases	Source
2011	Fenugreek sprouts	*Escherichia coli* O104	Multiple - Germany/France	3910 (55 deaths)	European Center for Disease Control, New Eng. J. Med. 2011;365:1763- 1780.

* The data presented were collected from several sources of information, such as peer-reviewed journals, newspapers, press releases, health units, national laboratory and government websites.

Appendix C: Analytical Methods Used for Microbial Analysis

Bacterial Analysis	Method Identification Number (Date Issued)*	Title of Method
***Salmonella* spp.**	MFLP-29** (July 2007, modified)	The Qualicon Bax® System Method for the Detection of Salmonella in a Variety of Food and Environmental Samples
	MFHPB-20 (March 2009)	Methods for the Isolation and Identification of *Salmonella* from Foods and Environmental Samples
***E. coli* O157:H7/NM**	MFLP-30 (May 2003, Supplement 1 May 2005 & Supplement 2 November 2006)	The Dupont Qualicon Bax® System Method for the Detection of *E. Coli* O157:H7 in Raw Beef and Fruit Juice
	MFLP-80 (March 2008)	Isolation of *E. coli* O157:H7 or NM in Foods
VTEC	CFIA and HC Published Methods[17, 18]	Detection of Verotoxin-Producing Escherichia coli in Food
		A Cloth-based Hybridization Array System (CHAS) for Identification of Priority Enterohemorrhagic *E. coli* in Food

* Published in the Compendium of Analytical Methods [21]

** MFLP-29 was performed as written with the following modification: Secondary enrichment was performed as outlined for cantaloupes, i.e., transferred from primary enrichment 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.