Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Targeted Survey

REPORT

2012/2013 – 2013/14 Targeted Surveys

Targeted Survey Investigating Bacterial Pathogens and Generic *E. coli* in Spices

RDIMS # 6332617

Canada

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Executive Summary

Targeted surveys are used by the Canadian Food Inspection Agency (CFIA) to focus its surveillance activities on areas of highest risk. The information gained from these surveys provides support for the prioritization of the Agency's activities in areas of greater concern and also scientific evidence to address areas of lesser concern. Targeted surveys have been incorporated into the CFIA's regular surveillance activities as a tool for generating essential information on certain hazards in foods, identifying/characterizing new and emerging hazards, highlighting potential contamination issues, assessing and promoting compliance with Canadian regulations, informing trend analysis, and prompting/refining human health risk assessments.

Spices, such as ground pepper, paprika, garlic, cinnamon and chili, are generally recognized as safe foods as they do not support the growth of bacterial pathogens due to their low moisture content. This assumption has been challenged in recent years by the occurrence of a number of outbreaks and recalls associated with contaminated spices in several countries. Bacterial pathogens can be introduced in spices through contaminated incoming ingredients or by cross-contamination during processing and can survive for extended periods of time in these products. The presence of bacterial pathogens in spices creates a potential risk for foodborne illness as spices can be used as seasonings in ready-to-eat foods, and in foods that would provide conditions suitable for bacterial growth.

In view of the above information, the CFIA has selected spices for enhanced surveillance. Over four years of targeted surveys (2010/11 to 2013/14) approximately 2,400 samples have been collected from Canadian retail locations and tested for the presence of bacterial pathogens of concern.

The main objectives of the 2012/13– 2013/14 targeted surveys reviewed in this report were to generate baseline surveillance data on the bacterial pathogens *Salmonella*, *Clostridium perfringens* (*C. perfringens*) and *Bacillus cereus* (*B. cereus*), as well as on generic *Escherichia coli* (*E. coli*), an indicator of fecal contamination, in a variety of spices available in the Canadian market. A total of 1,624 spice samples were collected and analyzed. The majority (99.4%) of the samples were assessed as satisfactory. Two samples (organic garlic and ground ginger) were assessed as unsatisfactory for the presence of *Salmonella*. The CFIA conducted appropriate follow-up activities, and both affected products were recalled. In addition to these, eight samples were assessed as investigative for elevated levels (10^4- 10^6 CFU/g) of *B. cereus*. Further evaluations were conducted on these samples, and one product was recalled. No reported illnesses were found to be associated with these contaminated products. The bacterial pathogen *C. perfringens* and generic *E. coli* were not found at levels of concern in any of the samples tested. These findings suggest that the spices sampled during this survey were predominantly

produced and handled under Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs).

The CFIA provides regulatory oversight of the food 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, including importers and retailers, is ultimately responsible for the safety of the food that they produce, import and sell, while consumers are responsible for the safe handling of the food they have in their possession. The CFIA will continue its surveillance activities and inform stakeholders of its findings.

1 Introduction

1.1 Targeted Surveys

The Canadian Food Inspection Agency (CFIA) monitors domestic and imported foods for the presence of allergenic, microbiological, chemical, and physical hazards. One of the tools used to maintain this oversight is targeted surveys, which are a means to establish baseline information on specific hazards and to investigate emerging risks. Targeted surveys are part of the Agency's core activities with other surveillance strategies such as the National Chemical Residue Monitoring Program (NCRMP), the National Microbiological Monitoring Program (NMMP), and the Children's Food Project (CFP). These surveys are complementary to CFIA surveillance activities in that they examine hazards and/or foods that may not be routinely included in national monitoring programs.

Targeted surveys are used to gather information on the possible occurrence or prevalence of hazards in defined food commodities. These surveys generate essential information on hazards in foods, identify or characterize new and emerging hazards, inform trend analysis, prompt or refine human health risk assessments, assess compliance with Canadian regulations, highlight potential contamination issues, and/or influence the development of risk management strategies.

Due to the vast number of hazard and food commodity combinations, it is not feasible, nor should it be necessary, to use targeted surveys to identify and quantify all possible hazards in all foods. To identify the food-hazard combinations with the greatest potential health risks, the CFIA uses a combination of scientific literature, media reports, and/or a risk-based model developed by the Food Safety Science Committee, a group of federal, provincial and territorial subject matter experts in food safety.

The targeted surveys reviewed in this report (2012/13 and 2013/14) represent a portion of the 2,400 spice samples that were collected between the fiscal years (April – March) 2010/11 and 2013/14. These surveys were designed to gather baseline information on the occurrence of bacterial pathogens of concern in spices available to Canadians at retail.

1.2 Codes of Practice, Acts, and Regulations

International food safety standards, codes of practice, and guidelines relating to food, food production and food safety are developed under the joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Codex Alimentarius Commission. Food producers and processors across the world are encouraged to follow these international standards. The *General Principles of Food Hygiene* (CAC/RCP 1-1969)[1] and the *Code of Hygienic Practice for Spices and Dried Aromatic Plants* (CAC/RCP 42-1995)[2] apply to the spices examined in this survey. 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 and processing of foods and food products, from primary production to packaging.

Spices available in the Canadian marketplace must comply with the *Food and Drugs Act* (FDA)[3] and *Food and Drug Regulations* (FDR)[4]. The FDA prescribes certain restrictions on the production, importation, sale, composition and content of foods and food products. Subsection 4(1)a of the FDA prohibits the sale of food contaminated with foodborne pathogens, and subsection 4(1)e and section 7 of the FDA prohibit the sale of unsafe food and food produced under unsanitary conditions.

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

2 Survey on Spices

2.1 Rationale

According to Agriculture and Agri-food Canada, spices are any dried plant product used primarily for seasoning[5] (e.g., ground pepper, paprika, garlic, cinnamon, chili). Spices have customarily been perceived as safe food products because their low moisture content prevents the growth of bacterial pathogens. However, a number of foodborne illness outbreaks linked to spices over the past two decades in different regions of the world[6] have prompted food safety experts to question the safety of these food ingredients in terms of microbial hazards. The majority of these outbreaks were associated with *Salmonella* and *Bacillus spp.* contamination (Appendix B).

Contamination of spices with bacterial pathogens can happen at various steps of production, from the introduction of contaminated incoming ingredients to cross-contamination during processing[7, 8]. Some pathogens such as *Salmonella* are able to survive for extended periods of time in spices[8]. In a US study on imported spices, it was determined that *Salmonella* can survive for at least 8 months[7]. Other pathogens such as *Bacillus cereus* (*B. cereus*) and *Clostridium perfringens* (*C. perfringens*), which can cause illness when ingested in large numbers, form spores that can survive for long periods of time in spices and are resistant to heat[9] (i.e., they can survive normal cooking temperatures). The risks associated with the presence of such bacterial pathogens in spices depend on the nature of the pathogen present and the manner in which the spice is used and consumed. For example, a contaminated spice added to a ready-to-eat food for flavour (e.g. paprika sprinkled on potato chips) could cause illness if a pathogen like *Salmonella* is present[7, 9]. The presence of high levels of spore-forming bacteria (> 10^6 CFU/g) such as *C. perfringens* or *B. cereus* in spices used in a cooking dish (e.g., casserole, stew) also represents a potential food safety concern as the spores can survive cooking and will grow in the prepared dish if held at room temperature[9].

Spore-forming bacteria *B. cereus* and *C. perfringens* are commonly found in spices[9], and the potential presence of *Salmonella* in spices has also been documented[7, 9]. In a study carried out by the British Health Protection Agency at retail and production premises in 2004, it was estimated that the prevalence of *Salmonella, B. cereus* (at levels $\geq 10^4$ cfu/g) and *C. perfringens* (at levels $\geq 10^3$ cfu/g) in dried spices and herbs in the United Kingdom (UK) was respectively 1%, 1% and 0.4%[10]. Irradiation is an effective treatment method to reduce the bacterial load in spices, and is permitted for use in a limited number of foods in Canada including spices[11]. However, irradiation is not permitted under the Canadian Organic Standards[12], and as a result cannot be used for the production of organic spices.

Based on the above information, spices have been selected for targeted surveillance. The objective of this surveillance was to generate baseline information on the occurrence of

Salmonella, *B. cereus*, *C. perfringens* and Generic *E. coli* in spices that are available to Canadians at retail.

2.2 Targeted Micro-organisms

2.2.1 Bacterial Pathogens of Concern

Salmonella is naturally found in the intestines of animals, such as poultry and cattle[13]. Most outbreaks associated with *Salmonella* are linked to the consumption of contaminated food of animal origin (e.g., chicken, raw milk). However, to a lesser extent, spices have also been identified as a potential vehicle for this pathogen[8]. Spices can become contaminated during processing from inadequately cleaned equipment or by using contaminated ingredients[9]. Ingredients can become contaminated during primary production in the field, by using improperly composted manure, contaminated water and/or wildlife feces, or during subsequent handling steps (sorting, drying, packaging)[1, 2].

C. perfringens and *B. cereus* are spore-forming and toxin-producing bacteria that are widely distributed in soil, dust and vegetation. Foods that are heavily contaminated by these bacteria (more than 10^6 organisms/g) can cause illness in humans[14]. It is estimated that *C. perfringens* and *B. cereus* are the second and fifth leading causes of foodborne illness in Canada[15]. Foods that have been associated with *C. perfringens* contamination include poultry, fish, vegetables, dehydrated foods (such as spices), and stews prepared with raw poultry or beef[14]. Foods that have been associated with *B. cereus* contamination include meats, milk, vegetables, fish, spices, sauces, rice, and starchy foods such as potatoes, pasta and cheese products[14, 13].

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

The *E. coli* bacteria that inhabit the large intestines of humans and animals are generally harmless. Due to their regular presence in the stools of humans and animals, the presence of *E. coli* in foods indicates direct or indirect contamination with fecal matter[16]. The presence of generic *E. coli* in foods can also indicate potential contamination with pathogenic enteric micro-organisms that also lives in the intestines of infectious humans and animals, such as *Salmonella*. It is important to note that the presence of generic *E. coli* in food only implies an increased risk for contamination with pathogenic microorganisms but does not conclusively indicate that these pathogenic organisms are present. High levels of generic *E. coli* in dry foods sold at retail indicate that contamination occurred at some point between primary production and final packaging.

2.3 Sample Collection

All samples were collected from national chain and local/regional grocery stores, as well as other conventional retail and natural food stores across Canada. The number of samples collected in the various geographic regions across Canada was based on the relative proportion of the population in the respective regions.

In this survey, a sample consisted of a single sampling unit (e.g., individual pre-packaged consumer-size package(s) from a single lot) with a total weight of at least 150g. This sampling approach is common for surveys conducted at retail, and is also used by other federal partners such as the Public Health Agency of Canada (PHAC) for the retail component of their FoodNet Surveys[17].

Samples were declared unfit for analysis if there were any issues with the conditions in which they were handled or shipped.

2.4 Analytical Methods and Assessment Guidelines

All samples were analysed using the analytical methods published in Health Canada's *Compendium of Analytical Methods for the Microbiological Analysis of Foods*[18] (Appendix C). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of the products sampled during this survey.

The assessment criteria presented herein (Table 3) are based on principles from the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*[19] and associated methods published in Health Canada's *Compendium of Analytical Methods*[18].

Table 3. Assessment Guidelines for bacterial pathogens and generic *E. coli* in Spices

Bacterial Analysis* (Method Identification Number)	Assessment Criteria		
	Satisfactory	**Investigative**	**Unsatisfactory**
***Salmonella* spp.** (MFLP-29 modified and MFHPB-20 if required for confirmation)	Absent in 25 g	N/A	Present in 25 g
Bacillus cereus (MFLP-42)	$\leq 10^4$ CFU/g	$10^4 < x \leq 10^6$ CFU/g	$> 10^6$ CFU/g
Clostridium perfringens (MFHPB-23)	$\leq 10^4$ CFU/g	$10^4 < x \leq 10^6$ CFU/g	$> 10^6$ CFU/g
Generic *E. coli* (MFHPB-19)	$\leq$ 100 MPN/g	100 < x $\leq$ 1,000 MPN/g	> 1,000 MPN/g

* *Compendium of Analytical Methods*[18].

Based on the current regulatory standards and microbiology testing criteria, samples can be assessed as “satisfactory,” “unsatisfactory” or “investigative.” Unsatisfactory samples 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). Samples assessed as investigative also require some form of follow-up activity, such as further sampling to verify the levels of generic *E. coli*, *B. cereus* or *C. perfringens* in the samples in question.

2.5 Survey Limitations

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

3 Results

3.1 Sample Distribution

A total of 1,624 spices were collected from retail and consisted of imported and domestic, conventionally and organically produced spices. Over 95% of the spices collected were imported (Table 1). There are currently no requirements in Canada to declare the country of origin on the label of imported pre-packaged products such as spices. Consequently, many of the imported spices sampled did not have the country of origin indicated on their label. These products were categorized under “unknown” in Table 1.

Table 1. Distribution of Samples by Country of Origin and Production Practice

Country of Origin	Organic Spices (Percent of total)	Conventional Spices (Percent of total)	Number of Samples (Percent of total)
Total Domestic (Canada)	1 (0.1%)	35 (2.2%)	36 (2.2%)
Total imported	76 (4.7%)	1,512 (93.1%)	1,588 (97.8%)
Unknown	14 (0.9%)	1,352 (83.3%)	1,366 (84.1%)
India	9 (0.6%)	41 (2.5%)	50 (3.1%)
United States	11 (0.7%)	23 (1.4%)	34 (2.1%)
China	3 (0.2%)	27 (1.7%)	30 (1.8%)
Sri Lanka	15 (0.9%)	5 (0.3%)	20 (1.2%)
Vietnam	2 (0.1%)	16 (1.0%)	18 (1.1%)
Spain	-	14 (0.9%)	14 (0.9%)
Israel	7 (0.4%)	5 (0.3%)	12 (0.7%)
Mexico	2 (0.1%)	7 (0.4%)	9 (0.6%)
Indonesia	5 (0.3%)	2 (0.1%)	7 (0.4%)
Egypt	5 (0.3%)	1 (0.1%)	6 (0.4%)
Hungary	-	4 (0.2%)	4 (0.2%)
Lebanon	-	3 (0.2%)	3 (0.2%)
South Africa	-	3 (0.2%)	3 (0.2%)
Trinidad and Tobago	-	3 (0.2%)	3 (0.2%)
United Kingdom	-	3 (0.2%)	3 (0.2%)
Peru	2 (0.1%)	-	2 (0.1%)
Taiwan	-	2 (0.1%)	2 (0.1%)
Norway	1 (0.1%)	-	1 (0.1%)
Thailand	-	1 (0.1%)	1 (0.1%)
Total	**77 (4.7%)**	**1,547 (95.3%)**	**1,624 (100.0%)**

Eleven types of spices (Table 2) were sampled for this survey, and included spices such as black pepper, paprika, garlic powder, cinnamon and chili powder. Most spices sampled during this survey were produced conventionally (95.3%) and a small percentage (4.7%) were produced organically.

Table 2. Distribution of Spices by Product Type and Production Practice

Product Type	**Organic Spices** (***Percent of total***)	**Conventional Spices** (***Percent of total***)	**Number of Samples** (***Percent of total***)
Black pepper	6 (0.4%)	292 (18.0%)	298 (18.3%)
Paprika	12 (0.7%)	230 (14.2%)	242 (14.9%)
Garlic powder	9 (0.6%)	211 (13%)	220 (13.5%)
Cinnamon	20 (1.2%)	181 (11.1%)	201 (12.4%)
Chili powder	3 (0.2%)	175 (10.8%)	178 (11.0%)
Curry powder	4 (0.2%)	120 (7.4%)	124 (7.6%)
Ginger	7 (0.4%)	114 (7.0%)	121 (7.5%)
Cumin	11 (0.7%)	74 (4.6%)	85 (5.2%)
Mustard	3 (0.2%)	60 (3.7%)	63 (3.9%)
White pepper	2 (0.1%)	52 (3.2%)	54 (3.3%)
Coriander	-	24 (1.5%)	24 (1.5%)
Turmeric	-	1 (1.1%)	1 (1.1%)
Cayenne pepper	-	13 (0.8%)	13 (0.8%)
Total	**77 (4.7%)**	**1,547 (95.3%)**	**1,624 (100%)**

3.2 Assessment Results

All of the spice samples were analysed for *Salmonella*, *C. perfringens*, *B. cereus* and generic *E. coli*, an indicator of fecal contamination. Most of these samples (99.4%) were assessed as satisfactory (Table 4). The bacterial pathogen *C. perfringens* and the indicator generic *E. coli* were not found at levels exceeding the satisfactory threshold in any of the samples.

Two samples were assessed as unsatisfactory due to the presence of *Salmonella*. These two products included a ground ginger product that was contaminated with *Salmonella* Paratyphi, and an organic cinnamon product that was contaminated with *Salmonella* Derby (Table 5). Further evaluations resulted in the recall of both products. Another eight samples were assessed as investigative for elevated levels of *B. cereus* (10^4 - 10^6 CFU/g) (Table 5). These samples included 3 garlic products, 2 ginger products, 2 curry powder products and 1 cinnamon product (Table 5). The CFIA initiated follow-up activities for these products which included additional testing and resulted in the recall of one of the contaminated curry powder product sampled.

Table 4. Summary of Results for Spices Analysed for *Salmonella* spp., *B. cereus*, *C. perfringens* and Generic *E. coli*

Method of Production	Origin	Number of Samples	Assessment		
			Satisfactory	Investigative	Unsatisfactory
Conventional	Domestic	35 (2.2%)	35 (2.2%)	0	0
	Imported	1512 (93.1%)	1504 (92.6%)	8 (0.5%)	1 (0.1%)
	Sub Total	1547 (95.3%)	1539 (94.8%)	8 (0.5%)	1 (0.1%)
Organic	Domestic	1 (0.1%)	1 (0.1%)	0	0
	Imported	76 (4.7%)	75 (4.6%)	0	1 (0.1%)
	Sub Total	77 (4.7%)	76 (4.7%)	0	1 (0.1%)
Total		**1,624**	**1,614 (99.4%)**	**8 (0.5%)**	**2 (0.1%)**

Table 5. Summary of Investigative and Unsatisfactory Results for Spices

Assessment Type	Method of Production	Country of Origin	Product Type	Reason for Assessment
Investigative	Conventional	Unknown	3 garlic products	B. *cereus* with levels of 7,500 CFU/g, 2,9000 CFU/g, and 26,000 CFU/g.
			2 ginger products	B. *cereus* with levels of 19,000 CFU/g and 29,000 CFU/g.
			Curry Powder	B. *cereus* with a level of 26,000 CFU/g.
		China	Curry Powder	B. *cereus* with a level of 94,000 CFU/g.
			Cinnamon	B. *cereus* with a level of 7,000 CFU/g.
Unsatisfactory	Conventional	Sri Lanka	Ginger	Contaminated with *Salmonella* Paratyphi
	Organic	Unknown	Garlic	Contaminated with *Salmonella* Derby

4 Conclusion and Discussion

In this survey (2011/12 – 2013/14), 99.4% of the spices analysed were assessed as satisfactory. In the remainder of the samples (0.6%), two samples were found to be contaminated with *Salmonella* (0.1%) and eight samples (0.5%) had elevated levels of *B. cereus* ($10^4 < x \leq 10^6$ CFU/g). The bacterial pathogen *C. perfringens* and bacteria generic *E. coli* were not detected at levels of concern in any of the samples tested.

The two *Salmonella* positive samples were assessed as unsatisfactory and triggered follow-up procedures including the recall of both affected products. The samples with elevated *B. cereus* levels were assessed as investigative and also triggered follow-up activities. These activities included further examinations and one product recall. It is important to note that there were no reported illnesses associated with the consumption of contaminated products sampled during this survey.

Overall, this survey suggests that the vast majority of spices sampled during this survey were produced and handled under good GAPs and GMPs. However, sporadic contamination with *Salmonella* and/or elevated levels of *B. cereus* can occur 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 consumers are responsible for the safe handling of food they have in their possession, the CFIA regulates the industry, provides oversight and promotes the safe handling of foods throughout the food production chain. The CFIA will continue its surveillance activities and will inform stakeholders of its findings.

5 References

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Appendix A: List of Acronyms and Abbreviations

B. cereus: *Bacillus cereus*

CFIA: Canadian Food Inspection Agency

CDC: Centres for Disease Control and Prevention

CFP: Children's Food Program

CFU/g: colony forming units per gram

C. perfringens: *Clostridium perfringens*

E. coli: *Escherichia coli*

FAO: Food and Agriculture Organization of the United Nations

FDA: Food and Drug Act

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

MFHPB: Microbiology Food Health Protection Branch

MFLP: Microbiology Food Laboratory Procedures

MPN: Most Probable Number

NCRMP: National Chemical Residue Monitoring Program

NMMP: National Microbiological Monitoring Program

PFGE: Pulsed Field Gel Electrophoresis

PHAC: Public Health Agency of Canada

spp.: species

UK: United Kingdom

US FDA: United States Food and Drug Administration

WHO: World Health Organization

Appendix B: Examples of Foodborne Disease Outbreaks Associated With Spices Contaminated with Microbial Pathogens (1993-2014)*

Year	Micro-organisms	Vehicle	Country	Cases	Source
1993	*Salmonella*	Paprika containing spice mixes and paprika-powdered potato chips	Germany	1,000	Giedon online & ProMed May 21, 2007 (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)
1995	*Bacillus subtis* & *Bacillus pumilis*	Turmeric on lamb seekh kebab	England	2	See reference #22
1996	*Salmonella* Enteridis	Black pepper	England	8	See reference #22
1997	*Bacillus subtilis*	Pepper (type not specified)	New Zealand	2	See reference #22
2002	*Salmonella* Braenderup	Curry powder used by caterers	England	20	CDR Archives (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)
2007	*Bacillus cereus*	Spice blend used in couscous dish	France	146	European Food Safety Authority 2007 (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)
2008	*Salmonella* Rissen	Ground white pepper imported, packaged and distributed by a California company	USA	87	Oregon Depart. of Human Services Public Health Division (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)
2009	*Bacillus cereus*	Rosepaprika (spice)	Denmark	48	The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2009 (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)

Year	Micro-organisms	Vehicle	Country	Cases	Source
2009-10	*Salmonella* Montevideo	Salami products with contaminated Imported Black and Red Pepper	USA	272	CDC (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)
2010	*Bacillus cereus*	White pepper	Denmark	112	EU 2010 report (Information provided by Judy D. Greig, Laboratory for Foodborne Zoonoses, PHAC)

* The data presented in the above table 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 (June 2012, modified)**	The Qualicon Bax® System Method for the Detection of *Salmonella* in a Variety of Food and Environmental Samples
	MFHPB-20 (March 2009)	Methods for the Isolation and Identification of *Salmonella* from Foods and Environmental Samples
B. cereus	MFLP-42 (May 2011)	Isolation and Enumeration of the *Bacillus cereus* Group in Foods
C. perfringens	MFHPB-23 (November 2001)	Enumeration of *Clostridium perfringens* in Foods
Generic *E. coli*	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in Foods

* All methods used are published *in the Compendium of Analytical Methods* (19)

** MFLP-29 was performed as written with the following modification: Secondary enrichment was performed as outlined for cantaloupes, i.e., transferred from buffered peptone broth as specified to RVS and TBG broths (Rappaport-Vassiliadis Soya Peptone broth and Tetrathionate Brilliant Green broth) and incubated for 24 ± 2 h at 42.5°C. After incubation 2 ml from each of RVS and TBG are combined to one sample and analysis proceeds at step 7.3.1.4 of the method.