Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2011/12 Targeted Surveys

Targeted Survey Investigating the Microbiological Quality and Safety of Bottled Drinking Water

RDIMS # 5462889

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 Canada, the annual consumption of bottled water has increased from 32.7 to 67.8 liter per person over the last decade. Bottled waters are not sterile and can contain bacteria from naturally occurring sources. These bacteria may lead to water spoilage, but generally do not cause waterborne illnesses. Harmful microorganisms could be introduced to bottled water during the production, transportation of source water and bottling, if Good Manufacturing Practices (GMPs) were not followed. Compared to other drinking water sources, very few outbreaks have been associated with bottled water. There has been no waterborne disease outbreaks associated with bottled water in Canada. However, with the significant increase in the consumption of bottled water, concerns over its microbiological safety and quality have been raised.

Considering the above factors and their relevance to Canadians, bottled water has been selected for a targeted survey in the 2011/12 fiscal year under the FSAP. The main objectives of this survey were to generate current baseline surveillance data on the microbiological quality and the occurrence of hazards of concern in bottled water available in the Canadian retail market.

In this survey, a total of 843 samples of bottled water, including imported and domestic, spring, mineral and other types of water, were collected and tested for bacterial, viral and parasitic microorganisms of concern. *Pseudomonas aeruginosa* and *Aeromonas hydrophila*, which can cause illness in people with low immunity, were not detected in any of the 504 samples sent for bacteriology analysis. One sample was found to exceed Health Canada's standard for total coliforms, a group of harmless bacteria used as an indicator of microbiological quality. *Cryptosporidium* was not detected in any of the 194 samples tested for this parasite. Hepatitis A virus was not detected in any of the 145 samples sent for virology testing, while Human Rotavirus and Norovirus were detected in one and two samples, respectively. It is important to note that the current methods for virus detection are molecular-based assays that do not differentiate living, infectious viruses from dead viruses. Therefore, it is impossible to determine if the viruses detected in the positive samples were capable of causing illness. No outbreaks associated with these products were reported during this survey. Based on the above considerations, immediate follow-up actions were not taken on the positive samples.

The Canadian Food Inspection Agency (CFIA) regulates and provides oversight to the food industry, works with provinces and territories, and promotes safe handling of bottled water throughout the production chain. However, it is important to note that the bottled water industry, importers, and retail sectors are ultimately responsible for the bottled water they produce, import, and sell, while individual consumers are responsible for the safe handling of the bottled water they have in their possession. Moreover, general advice for storage and safe handling of bottled water is 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 (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.

Within the current regulatory framework, some commodities, such as meat products, traded internationally and inter-provincially are regulated by specific Acts and Regulations. These commodities are referred to as federally registered commodities. On the other hand, commodities that are regulated solely under the *Food and Drugs Act* [3] and *Regulations* [4] are the non-federally registered commodities. These non-federally registered commodities encompass 70% of the food available for sale in Canada, including imported and domestic food products. Targeted surveys are primarily directed towards non-federally registered commodities. Bottled waters belong to the non-federally registered food category.

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. 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.

This targeted survey represents 843 samples of bottled drinking water collected in 2011/12 to gather information on the microbial quality and the occurrence of bacterial pathogens, viruses and parasites of concern in bottled water 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 Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Codex Alimentarius Commission. Producers of bottled water are encouraged to follow these international codes of practice. Of relevance for this survey are the *Code of Hygienic Practice for Bottled/Packaged Drinking Waters* (CAC/RCP 48-2001) [5] and the *Recommended International Code of Hygienic Practice for the Collecting, Processing and Marketing of Natural Mineral Waters* (CAC/RCP 33-1985) [6]. These codes recommend general techniques for collecting, processing, packaging, storing, transporting, distributing, and offering for sale a variety of drinking waters for direct consumption. These codes are recommended to be used in combination with the *Recommended International Code of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969, Rev. 4-2004) [7], which addresses hygienic practices to control and reduce the potential for contamination with microbial, chemical, and physical hazards at all stages of the production of foods and food products from primary production to packaging.

In Canada, bottled drinking water is considered to be a food product and must comply with the *Food and Drugs Act* (FDA) [3] and the *Food and Drug Regulations* (FDR) [4], 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. In particular, Division 12 of the FDR provides definitions and specific microbiological standards and guidelines for the different types of bottled water (spring water, mineral water, and water in sealed containers).

The FSAP targeted surveys are primarily conducted for surveillance and not for regulatory compliance verification 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 health risk assessments. Depending on the findings of the investigation, a recall of the affected product may be warranted.

2 Survey on Bottled Water

2.1 Rationale

In Canada, the annual per capita consumption of bottled water has increased from 32.7 liters to 67.8 liters from 1999 to 2009 [8]. The sales are expected to reach an average growth rate of 1% over the period of 2010 to 2015 [8]. With the significant increase in the consumption of bottled water, concerns over its microbiological safety and quality have been raised.

Studies on the microbiological quality of various types of bottled water on the Canadian market in the 1990's found that 2% of the samples had total coliforms exceeding the standard for bottled waters, and that 5% of the samples had aerobic colony counts (ACC) exceeding the limit set for bottled waters sampled within 24 hours of packaging [9].

The source water used to manufacture bottled water can be from underground spring water and mineral water, or other water sources that have been approved to be safe and of good sanitary quality with or without treatment. Bottled waters are not sterile and can contain bacteria from naturally occurring sources. In addition to these indigenous microflora, harmful microorganisms could be introduced to bottled water during the production, transportation of source water and bottling, if GMPs were not followed [10,11]. However, compared to other drinking water sources, very few outbreaks have been associated with drinking bottled water [12, 13]. There has been no waterborne disease outbreaks associated with drinking bottled water in Canada. In the last decade, there were several recalls of bottled water in Canada due to microbiological safety concerns (Appendix B). These recalled bottled waters were contaminated *Pseudomonas aeruginosa* (*P. aeruginosa*), an opportunistic pathogen considered to be a risk to immunocompromised patients and infants.

Based on the above information, bottled water has been selected for targeted surveillance under FSAP in 2011/12. The overall objective was to gather baseline information on the microbiological quality and the occurrence of pathogens of concern in bottled water available to Canadians at retail.

2.2 Targeted Microorganisms

2.2.1 Aerobic Bacteria and Coliforms as Water Quality Indicators

Aerobic bacteria are generally harmless bacteria and are normal components of the environment that can be found from soil and natural water sources. The total aerobic bacteria (aerobic colony count (ACC)) is used to assess the general bacterial content of bottled water. High counts of ACC may lead to spoilage of the water, but they are not a good indicator of the sanitary conditions and practices at the time of bottling. Only when the ACC is determined within 24 hours of bottling (before growth of indigenous bacteria), can a high count indicate poor GMPs during the processing of bottled water [14].

Coliforms are a group of generally harmless bacteria that are used as an indicator to assess the hygiene status of bottled water. Coliform bacteria can be found in the environment (water and soil) as well as in human and animal feces. Therefore, the presence of coliforms in bottled water does not necessarily indicate that fecal contamination occurred. However, the presence of coliforms in bottled natural mineral water indicates that contamination occurred during the bottling process, as qualified natural mineral water sources should be free of coliforms [6]. Nonetheless, a high level of coliforms in bottled water is an indication of deterioration in microbiological quality (e.g., poor quality of the water source, ineffective water treatments, or possible contamination during the bottling process).

2.2.2 *Pseudomonas aeruginosa and Aeromonas hydrophila.*

Pseudomonas aeruginosa (*P. aeruginosa*) and *Aeromonas hydrophila (A. hydrophila)* are opportunistic bacterial pathogens that widely exist in the environment. *P. aeruginosa* is commonly found in feces, soil, surface water, or organic material in contact with water. The presence of *P. aeruginosa* in bottled water can indicate fecal contamination from humans and/or farm animals or poor GMPs during bottling. In contrast, *A. hydrophila* show no particular association with fecal contamination. Both *P. aeruginosa* and *A. hydrophila* can cause diarrhoea and can be life threatening for immunocompromised patients or infants. Therefore, these opportunistic bacterial pathogens are considered to be a risk to immunocompromised patients and infants, but do not appear to be a risk for the general population.

2.2.3 Viral Pathogens

Viruses are nucleic acid molecules that can only replicate inside host cells but can survive in aquatic environment. Source water (usually ground water) can be contaminated through polluted storm water or floodwater containing viruses [11, 15]. As viruses are much smaller in size than bacteria, they can escape filtration barriers designed to remove bacteria from source water. If other methods of water treatment fail to eliminate contaminated viruses, they can survive in the water and cause waterborne illnesses [16].

Norovirus (NoV), hepatitis A virus (HAV), and human rotavirus (HRV) are enteric viruses that can be transmitted through contaminated water and cause waterborne illnesses. Generally, NoV causes acute gastroenteritis without long term effects. HRV also causes acute gastroenteritis, but illness may be much more serious in very young children, elderly, and people with weak immune system. HAV causes hepatitis A, an infectious liver disease that is generally self-limiting, but with possible severe outcomes of fulminant hepatitis [17].

There are currently five recognized NoV genogroups (GI to GV); Genotypes I and II (GI and GII) are known to be responsible for most human illnesses [17]. For HRV, three of the six serological groups (A, B, and C) identified can infect humans, and group A is the most common cause of infections [17].

2.2.4 Parasitic Protozoan – Cryptosporidium

Certain types of intestinal protozoa pathogenic to humans, such as *Cryptosporidium*, mainly spread through drinking water. *Cryptosporidium* can infect humans as well as domestic animals including cattle and sheep. Thus, there are more opportunities for its oocysts to spread, as they are excreted from infected human and animal feces into the environment including aquatic environments. The oocyst is the infectious stage of *Cryptosporidium* with a protective wall that facilitates its survival in aquatic environment and that is resistant to chemical treatment (e.g., iodine, chlorine). However, oocysts can be removed by the filtration treatment of the water source.

Human infection (cryptosporidiosis) occurs by ingestion of infective oocysts. *Cryptosporidium* can complete its life cycle within a single host, resulting in oocysts that are excreted in its host feces and are capable of transmission to a new host. Cryptosporidiosis is self-limiting in most cases, but it is a particular risk to immunocompromised patients with the possibility of fatal outcome [17].

2.3 Sample Collection

Bottled drinking waters that were packaged in plastic or glass water bottles with a volume of about 500 mL were collected from national chain and local/regional grocery stores and other conventional retail located in 11 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 year round (from April 1st, 2011, to March 31st, 2012).

In this survey, a sample consisted of one consumer size bottled water (about 500 mL in volume). Large format of bottled waters (18 litters) were excluded from this survey.

2.4 Sample Distribution

A total of 843 bottled water samples were collected, including 495 (58.7%) samples of domestically produced and 321 (38.1%) samples of imported bottled water, as well as 27 (3.2%) samples with no information on the country of origin (Table 1).

The samples are grouped in three categories: mineral water, spring water and other bottled water (i.e., water in sealed containers) (Table 2). They differ mainly by the type of source water and the additional treatments they are subjected to. Spring water and mineral water are from natural underground sources and may or may not be treated (e.g., carbonation or ozonation). Bottled water that is not labelled as spring or mineral water may be from other water sources (e.g., municipal tap water) and may be treated to make it fit for people to drink or to modify its original composition (mineralized or demineralized).

As indicated in Table 2, samples included mineral water (3.1%), spring water (59.4%), and other bottled water (37.5%). In terms of additional treatment, carbonation or oxygenation treated water accounted for 8% of the samples.

Table 1 Sample Distribution by Country of Origin

Country of Origin	Samples Tested for:			Total	
	Bacteria	Viruses*	Parasites*		
	Number of Samples	Number of Samples	Number of Samples	Number of Samples	Percentage of Samples
Canada	294	86	115	495	58.7
Subtotal - Domestic	294 (58.3%)	86 (59.3%)	115 (59.3%)	495	58.7
Argentina	0	1	0	1	0.1
Croatia	2	0	1	3	0.4
Fiji	46	16	22	84	10.0
France	64	20	26	110	13.0
Germany	1	0	0	1	0.1
Greece	0	0	1	1	0.1
Hong Kong	1	0	0	1	0.1
Iceland	7	5	2	14	1.7
Italy	21	4	10	35	4.2
Micronesia	1	0	1	2	0.2
New Zealand	1	1	0	2	0.2
Norway	4	2	0	6	0.7
Poland	4	1	0	5	0.6
Portugal	6	3	3	12	1.4
Romania	2	0	0	2	0.2
Russia	1	0	0	1	0.1
Samoa	1	0	1	2	0.2
Slovenia	4	0	0	4	0.5
United Kingdom	2	2	2	6	0.7
United States	20	4	5	29	3.4
Subtotal- Imported	188 (37.3%)	59 (40.7%)	74 (38.1%)	321	38.1
Unknown**	22	0	5	27	3.2
Total	**504**	**145**	**194**	**843**	**100.0**

* The number of samples for these tests was limited by the laboratory testing capacity.

** Products had no information on country of origin.

Table 2 Sample Distribution by Water Types

Type of Water	Samples Tested for: Bacteria	Viruses	Parasites	Total	
	Number of Samples	Number of Samples	Number of Samples	Number of Samples	Percentage of Samples
Mineral water					
- **Mineral**	8	3	2	13	1.4
- **Carbonated**	13	1	0	14	1.7
Subtotal – mineral water	21	4	2	27	3.1
Spring water					
- **Spring**	279	79	114	472	56.0
- **Carbonated**	11	2	1	14	1.7
- **Oxygenized**	12	1	1	14	1.7
Subtotal – spring water	302	82	116	500	59.4
Other bottled water*					
- **Regular**	25	25	9	59	7.0
- **Carbonated**	0	1	1	2	0.2
- **Oxygenized**	20	4	2	26	3.1
- **Mineralized**	40	8	23	71	8.4
- **Demineralized**	66	16	29	111	13.2
- **Glacial water**	19	3	6	28	3.3
- **Artesian water**	11	2	6	19	2.3
Subtotal – Other bottled water	181	59	76	316	37.5
Total	**504**	**145**	**194**	**843**	**100**

* other bottled water: samples of bottled water that were not labelled as spring water or mineral water were considered as other bottled water (i.e., water in sealed containers)

2.5 Method Details

For bacterial analysis, 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 D). These methods are used for regulatory testing by the CFIA and are fully validated for the analysis of water samples.

Samples were analyzed for Hepatitis A virus, Norovirus (GI and GII) and Rotavirus using modified versions of methods published in Health Canada's *Compendium of Analytical Methods* for the Microbiological Analysis of Foods [18] (Appendix D). Samples were first screened by reverse-transcriptase Polymerase Chain Reaction (RT-PCR). Samples that screened positive by RT-PCR were further characterized by cloning and sequencing to confirm the presence of the targeted virus. Confirmed positive results were re-analyzed by real-time RT-quantitative PCR (RT-qPCR) to estimate the number of viral genomic copies. Results were reported as "detected" when the virus' genetic material was detected and confirmed, and as "not detected" when it was either not detected or not confirmed.

For *Cryptosporidium* analysis, water samples were first concentrated by centrifugation. The resulting pellet was subjected to DNA extraction and a real-time polymerase chain reaction assay (q-PCR) with melting curve analysis (MCA) [19, 20]. Any samples that had qPCR products and a melting temperature similar to the *Cryptosporidium* controls would be cloned and sequenced for confirmation. Results were reported as "detected" when the parasite's genetic material was detected and confirmed by sequencing, and as "not detected" when it was either not detected or not confirmed.

It is important to note that the PCR-based methods used for detecting viruses and parasites do not differentiate live from dead organisms. The viruses and parasites analysed during this survey cannot be cultured *in vitro*, and as such, the viability of these microorganisms and the potential for infection cannot be assessed.

2.6 Assessment Guidelines

The assessment criteria presented below (Table 3 & 4) are based on the principles of the *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods* [21] and associated methods published in Health Canada's *Compendium of Analytical Methods* [18].

Table 3 Assessment Guidelines for Opportunistic Bacterial Pathogens in Bottled Water Samples

Bacterial Analysis*	Assessment Criteria	
	Satisfactory	Unsatisfactory
Pseudomonas aeruginosa (MFLP-61B)	Absent in 100 mL	Present in 100 mL
Aeromonas hydrophila (MFLP-58B)	Absent in 100 mL	Present in 100 mL

* Compendium of Analytical Methods [18].

Table 4 Assessment Guidelines for Quality Indicators in Bottled Water Samples

Bacterial Analysis*	Assessment Criteria			
	Satisfactory	Investigative		Unsatisfactory
Total Coliforms (MFHPB-19)	≤ 1.8 MPN/100 mL	1.8 < x ≤ 10 MPN/100 mL		> 10 MPN/100 mL
ACC (MFHPB-18)**	<100 CFU/mL	$10^2 < x \leq 10^4$ CFU/mL (Elevated level)	$>10^4$ CFU/mL (High level)	N/A

* *Compendium of Analytical Methods* [18].

** Testing for ACC should technically be done within 24 hours of bottling to be able to assess GMPs and compliance with the regulations [22].

Unsatisfactory assessments for the opportunistic bacterial pathogens would have been subject to immediate follow-up actions, such as directed follow-up sampling, inspection of establishment, health risk assessment, and/or product action (e.g., product recall).

Investigative and unsatisfactory assessments for quality indicators in bottled water did not require immediate follow-up activities (Table 4). As the samples were collected at retail, the ACC analysis could not be done within 24 hours of bottling as recommended by Health

Canada to evaluate GMPs and verify compliance with the regulations [22]. The assessment criteria used by the CFIA for ACC were set for the purpose of gathering current information on the general bacteria contents of bottled water in the Canadian market.

There are currently no internationally recognized assessment criteria and harmonized analytical methods for the detection of viruses or parasites. The current methods for virus and parasite detection are molecular-based assays that do not differentiate living, infectious viruses from dead viruses. It is impossible to determine whether the viruses in positive samples were capable of causing illness based on the laboratory results alone. In the absence of epidemiological information linking the positive samples to illnesses, the CFIA did not take immediate follow-up actions on these samples. However, the CFIA will continue to monitor the situation and will use the results to inform the bottled water industry of potential contamination issues.

2.7 Limitations

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

Given the varying channels of commerce, the source of the products can change dramatically from one year to the next. As such, there was an insufficient number of samples in this study to carry out a detailed analysis of the results based on country of origin. In cases of positive results, positive rates between countries are not considered to be statistically comparable.

3 Results

3.1 Bacteriology Results

A total of 504 samples of bottled water were tested for opportunistic bacterial pathogens *P. aeruginosa* and *A. hydrophila,* and indicators of water quality total coliforms and ACC. No opportunistic bacterial pathogens were found in any of the samples tested. One sample (0.2%) of imported bottled spring water was found to contain a high level of total coliforms (600 MPN/100 mL) and was assessed as unsatisfactory (Table 5).

A total of 71 samples (14.1%) were assessed as investigative (Table 5). These samples included mineral and spring water with elevated or high levels of ACC and other bottled water (i.e., non mineral/spring water in sealed container) with elevated levels of ACC.

Table 5 Summary of Bacterial Analysis Results

Product	Number of Samples	Assessment*		
		Unsatisfactory	Investigative	Satisfactory
Mineral water	21	0	2	19
Spring water	302	1	57	244
Other bottled water	181	0	12	169
Total	504 (100%)	1 (0.2%)	71 (14.1%)	432 (85.7%)

High levels of ACC (>10^4 CFU/mL) were found in 14 samples (2.7%). All these samples were spring water (Table 6).

Table 6 Summary of Samples with High Levels of ACC

Product Type /Country of Origin	**High levels of ACC ($>10^4$ CFU/mL)**
Natural spring water /Canada	7.5×10^4 CFU/mL
Spring water /Canada	6.7×10^4 CFU/mL
Natural spring water /Canada	6.4×10^4 CFU/mL
Spring water /Canada	4.1×10^4 CFU/mL
Natural spring water /Canada	4.0×10^4 CFU/mL
Natural spring water /Canada	3.8×10^4 CFU/mL
Natural spring water /Canada	2.4×10^4 CFU/mL
Natural spring water /Canada	1.9×10^4 CFU/mL
Spring water /USA	1.9×10^4 CFU/mL
Natural spring water /Iceland	1.8×10^4 CFU/mL
Spring water /Fiji	1.7×10^4 CFU/mL
Natural spring water /Canada	1.3×10^4 CFU/mL
Natural spring water /Canada	1.1×10^4 CFU/mL
Spring water /Fiji	1.1×10^4 CFU/mL

3.2 Virology Results

A total of 145 samples were tested for their potential contamination with viruses. HAV was not detected in any of the samples. NoV was detected in two samples (1.4%) and HRV was detected in one sample (0.7%) (Table 7 & 8).

Table 7 Summary of Viral Analysis Results

Product	Number of Samples	Results (HAV, HRV, and NoV)	
		Detected	Not Detected
Mineral water	4	0	4
Spring water	82	2	80
Other type water	59	1	58
Total	145 (100%)	3 (2.1%)	142 (97.9%)

Table 8 Summary of Positive Samples

Product Type /Country of Origin	Positive Samples
Natural spring water /Iceland	NoV GI
Natural spring water /Italy	NoV GII
Glacial water/Canada	HRV type A

The positive results indicate that the water came in contact with the virus at some point before bottling. The available methods for virus detection are molecular-based assays which do not differentiate living/infectious viruses from dead viruses. There were no reported outbreaks related to drinking bottled water in Canada during the survey. Thus there were no immediate follow-up actions taken from the CFIA.

3.3 Parasitology Results

A total of 194 samples were tested for their potential contamination by *Cryptosporidium* oocysts. *Cryptosporidium* was not detected in any of the samples (Table 9).

Table 9 Summary of *Cryptosporidium* Analysis Results

Product	Number of Samples	Assessment	
		Detected	Not Detected
Mineral water	2	0	2
Spring water	129	0	129
Water in sealed containers	63	0	63
Total	194 (100%)	0 (0%)	194 (100%)

4 Discussion

In this survey (2011/12), 843 samples of bottled water were collected and tested for opportunistic bacterial pathogens of concern and microbiological quality (504 samples), viruses (145 samples) and a parasite of concern (194 samples).

In terms of microbiological safety, the results indicate that the opportunistic bacterial pathogens *P. aeruginosa* and *A. hydrophila*, the parasite *Cryptosporidium*, and the virus HAV were not found in any of the samples tested. The viruses HRV and NoV were detected in one and two samples, respectively. No HRV or NoV outbreaks associated with bottled water were reported in Canada during this survey. Since the laboratory results were not a direct evidence of a health risk, the CFIA did not take immediate follow-up actions on these products.

In terms of microbiological quality, the results indicate that only one sample (0.2%) did not meet the Canadian standard for total coliforms, and that 14 samples (2.7%) had ACC levels exceeding the limit of 10^4 CFU/mL. All the samples that exceeded the ACC limit were spring waters. The results of this survey suggest that the microbiological quality of bottled water in the Canadian market has improved over the time. Early studies of bottled water in the Canadian market in the 1990's found that 2% (compared to 0.2% in the current study) of various types of bottled water sampled had total coliforms exceeding the standard, and that 5% (compared to 2.7% in the current study) of the samples had ACC exceeding the limit of 10^4 CFU/mL[11]. Several studies have suggested that general bacterial contents (or ACC) can increase rapidly after the water is bottled and can increase after storage at room temperature [14, 23]. Additional disinfection step in water manufacturing process, such as carbonation/ozonation, may further improve the microbiological quality of bottled water.

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

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Appendix A: List of Acronyms

ACC: aerobic colony counts

CDC: Centres for Disease Control and Prevention

CFIA: Canadian Food Inspection Agency

FAO: Food and Agriculture Organization of the United Nations

FDA: *Food and Drugs Act*

FDR: *Food and Drug Regulations*

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

GMPs: Good Manufacturing Practices

HAV: Hepatitis A virus

HRV: Human Rotavirus

HC: Health Canada

MPN: Most Probable Number

NoV: Norovirus

PHAC: Public Health Agency of Canada

USFDA: United States Food and Drug Administration

WHO: World Health Organization

°C: Degree Celsius

g: gram

Appendix B: Bottled Water Recalls in Canada (2000 - 2012)

List Number	Year-Month	Country of Origin	Microorganism of Concern	Class of Recall	Type of Bottled Water
1	2000-12	Canada	*Coliforms*, 1600 MPN/100 mL	Class III	Flavored bottled water
2	2001-04	Canada	*P. aeruginosa*	Class II	Spring water (18.9L)*
3	2001-04	Canada	*P. aeruginosa*	Class II	Spring water (18.9L)*
4	2001-04	Canada	*P. aeruginosa*	Class II	Spring water (500 mL)*
5	2002-03	USA	*P. aeruginosa*	Class II	Natural spring water (500 mL)
6	2002-07	Canada	*P. aeruginosa*	Class II	Natural spring water (500 mL)
7	2002-10	Canada	*P. aeruginosa*	Class II	Bottled water (18L)
8	2004-01	Canada	*P. aeruginosa*	Class II	Natural spring water (500 mL)
9	2004-01	Canada	*P. aeruginosa*	Class II	Natural spring water (18.9L)
10	2004-06	Canada	*P. aeruginosa*	Class II	Natural artesian spring water (18.9L)
11	2009-02	Canada	*P. aeruginosa*	Class II	Bottled water (11.5L)
12	2009-10	Canada	*Coliforms*, 160 MPN/mL	Class II	Bottled distilled water (18.9L)
13	2012-08	Canada	Inadequate process control	Class III	Bottled distilled water (4 L)

* these recalls are related

Appendix C: Analytical Methods Used for Microbial Analysis

Bacterial Analysis	Method Identification Number (Date Issued)	Title of Method*
Aerobic Bacteria	MFHPB-18 (Oct 2001)	Determination of the Aerobic Colony Count in Foods
Coliforms	MFHPB-19 (April 2002)	Enumeration of Coliforms, Faecal Coliforms and of *E. coli* in Foods Using the MPN Method
Pseudomonas aeruginosa	MFLP-61B	Enumeration of *Pseudomonas aeruginosa* in Prepackaged Ice and Water in Sealed Containers by the Hydrophobic Grid-Membrane Filter (HGMF) Technique
Aeromonas hydrophila	MFLP-58B	Enumeration of *Aeromonas hydrophila* in Ice and Water by the Hydrophobic Grid-Membrane Filter (HGMF) Technique
Hepatitis A Virus	CFIA-VAD-02	Method to Concentrate and Purify Viruses of Clinical Interest from Food Using Magnetic Cationic Beads.
	CFIA-VAD-04 (internal, modified version of OLFP-07*)	Detection of HAV using conventional RT-PCR
Norovirus (GI and GII)	CFIA-VAD-02	Method to Concentrate and Purify Viruses of Clinical Interest from Food Using Magnetic Cationic Beads.
	CFIA -VAD-06 (internal, modified version of OLFP-10)	Detection of Norovirus GI using conventional RT-PCR
	CFIA -VAD-07 (internal, modified version of OLFP-10)	Detection of Norovirus GI using real time RT-PCR
	CFIA -VAD-12 (internal, modified version of OLFP-10)	Detection of Norovirus GII using conventional RT-PCR
	CFIA-VAD-11 (internal, modified version of OLFP-10)	Method for cloning, sequencing and molecular characterization of viral genomic fragments amplified by molecular methods
Rotavirus	CFIA-VAD-02	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 OLFP-10*)	Method for cloning, sequencing and molecular characterization of viral genomic fragments amplified by molecular methods
Cryptosporidium	CFAP-M-0016	Processing of produce, bottled water, and apple cider for *Cyclospora* or *Cryptosporidium* testing
	CFAP-M-0018	Detection and differentiation of protozoan oocysts by real-time quantitative PCR and melt curve analysis (qPCR-MCA)

**Compendium of Analytical Methods* [19].