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

Food Safety Action Plan

REPORT

2011/12-2012/13 Targeted Surveys

Targeted Survey Investigating *Cyclospora cayetanensis* and *Cryptosporidium* spp. in Fresh Produce

RDIMS # 5946461

Canada

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Executive Summary

The Food Safety Action Plan 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.

Cyclospora and *Cryptosporidium* are protozoan parasites which infect humans, primarily through contaminated food and water. *Cyclospora* is endemic in a number of subtropical and tropical countries. *Cryptosporidium* infection can be found in people worldwide. *Cyclospora* and *Cryptosporidium* infections can cause mild to severe gastrointestinal (GI) symptoms including, but not limited to, diarrhoea, weight loss, cramping, flatulence, nausea, fatigue and low grade fever.

Cyclospora and *Cryptosporidium* were ranked 13th and 5th, respectively, out of 24 parasites in overall global ranking for their public health importance by a Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) expert committee (September 3 to 7, 2012). Produce such as fresh herbs and berries have been identified in the past as sources of *Cyclospora* and *Cryptosporidium* contamination in Canada. This survey focussed on fresh herbs, berries, green onions and mushrooms.

The objective of this survey was to determine the occurrence and distribution of *Cyclospora* and *Cryptosporidium* contamination in fresh produce such as herbs, berries, mushrooms and green onions. A total of 1590 samples were analyzed for the presence of *Cyclospora* and 1788 samples were analyzed for *Cryptosporidium*. Samples were collected at retail from various regions across Canada between May 2011 and March 2013.

Of the samples analyzed for *Cyclospora*, none were positive for the parasite. Of the samples analyzed for *Cryptosporidium*, six samples of green onions, one sample of parsley, and one sample of mushroom were positive, however, the analytical method used to detect the parasites in the samples cannot determine if the parasite is viable and potentially infectious. It is important to note that there were no reported illnesses associated with the consumption of the products found to be positive for *Cryptosporidium*. Positive results are followed up by the Canadian Food Inspection Agency (CFIA). In this case, because of the perishable nature of the products and the time elapsed between sample pick up and the completion of analysis, the fresh product was no longer available on the market when the parasite was detected. As such, no direct follow up was possible. This information was used to inform CFIA's programs and inspection activities.

The Canadian Food Inspection Agency regulates and provides oversight to the industry, works with provinces and territories, and promotes safe handling of foods throughout the food production chain. However, it is important to note that the food industry and retail sectors in Canada are ultimately responsible for the food they produce and sell, while individual consumers are responsible for the safe handling of the food they have in their possession. Moreover, general advice for the consumer on the safe handling of foods is widely available. 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's) Food Safety Action Plan (FSAP) [2] 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 is 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 and/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 (FSSC), a group of Canadian federal, provincial and territorial subject matter experts in the area of food safety [3].

This survey was designed to gather baseline information between May 2011 and March 2013 on the occurrence of *Cyclospora* and *Cryptosporidium* on fresh herbs, berries, mushrooms, and green onions 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/World Health Organization (FAO/WHO) Codex Alimentarius Commission. Producers of fresh fruits and vegetables 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) [4] and the *Recommended International Codes of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969) [5]. These codes address Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) which, when applied, control and reduce the potential for contamination with microbial, chemical, and physical hazards at all stages of production of fresh fruits and vegetables, from primary production to packaging.

Fresh fruits and vegetables available in the Canadian market must comply with the *Food and Drugs Act* (FDA) [6] and the *Food and Drug Regulations* (FDR) [7], which prescribe certain restrictions on the production, importation, sale, composition and content of foods and food products. Section 4(1)a of the FDA prohibits the sale of food contaminated with foodborne pathogens, while sections 4(1)e and 7 prohibit the sale of unsafe food and food produced under unsanitary conditions.

Fresh fruits and vegetables sold in Canada must also comply with the safety requirements of the *Fresh Fruit and Vegetable Regulations* [8] under the *Canada Agricultural Products Act* [9]. 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 portions of the FDA and FDR are enforced by the CFIA.

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 a food safety investigation, including activities such as follow up sampling, inspections of facilities and health risk assessments. Depending on the findings, a recall of the affected product may be warranted.

2 Survey on Fresh Produce

2.1 Rationale

Cyclospora cayetanensis and *Cryptosporidium* spp. are food- and waterborne parasites. While *Cyclospora* is endemic in a number of subtropical and tropical regions of the world [10], *Cryptosporidium* can be found in both developed and developing countries worldwide with varying levels of prevalence [11, 12].

Human infection occurs through the ingestion of fresh produce or water contaminated with faeces (of human origin in the case of *Cyclospora cayetanensis*) that contain the oocysts. The oocyst is a structure that can survive long periods of time outside the host, when excreted by an infected individual.

The oocysts of *Cyclospora* need specific environmental conditions, including warm temperatures, to undergo sporulation and become infective. Once sporulated and ingested by a person, the infective *Cyclospora* oocysts reach the gastrointestinal tract where they release sporozoites that penetrate the lining of the small intestine (epithelial cells). Inside the epithelial cells, the sporozoites go through asexual multiplication and sexual development, upon which they are excreted in faeces as mature oocysts [13 14]. The life cycle of *Cryptosporidium* is similar to that of *Cyclospora,* however, one of the main differences between the two parasites is that *Cryptosporidium* oocysts are infectious upon excretion into the environment.

Infection with *Cyclospora* or *Cryptosporidium* may cause a number of gastrointestinal symptoms such as diarrhoea, loss of appetite, weight loss, bloating, cramping, increased flatulence, nausea, fatigue and low grade fever. The severity of these symptoms depends on the number of oocysts ingested and the condition of the host at the time of infection [14]. Trade of fresh produce with countries having high rates of cyclosporosis (*Cyclospora* infection) has increased the potential that Canadians may be exposed to *Cyclospora* [15]. *Cryptosporidium* is prevalent worldwide and was reported in 2004 to have infected approximately 4% of the North American population [12].

As a result of a FAO/WHO expert meeting (September 3 to 7, 2012), where parasites were ranked primarily based on their impact on disease burden, *Cryptosporidium* was ranked 5th and *Cyclospora* was ranked 13th out of 24 parasites in overall global public health importance [16].

Fresh produce have been reported to be responsible for numerous outbreaks of foodborne illness. Following a joint FAO/WHO expert meeting concerning microbial hazards in fresh fruits and vegetables, and as a result of a ranking process applied to identify commodities

that pose the greatest concern for contamination, herbs were deemed as a level one priority while berries and green onions were deemed as a level two priority [17]. From 1995 to 2013, there were 28 documented outbreaks in North America (including 8 in Canada) associated with herbs (and products made with herbs) and berries contaminated with *Cyclospora* (Appendix B) and one outbreak associated with leafy herbs contaminated with *Cryptosporidium*. Leafy herbs were identified as one of the top five produce commodities attributed to increased produce-associated foodborne disease outbreaks in the U.S. from 1998 to 2006 and in 1996, berries were among the main commodities that caused a large scale foodborne outbreak of cyclosporosis in the U.S. and Canada [15].

Fresh produce may become contaminated with *Cyclospora* or *Cryptosporidium* in the field as a result of contaminated water being used for irrigation or for mixing fertilizers. *Cyclospora* or *Cryptosporidium* may also be present in water used for washing produce or during the processing and packaging of the product [14]. Contaminated fingers of workers during harvest may also be a source of the parasites. The oocysts may be trapped by the irregularly shaped surfaces of some produce such as raspberries and fresh herbs. Raspberries are particularly problematic, in that they also have hair that are resistant to breakage, even from washing, that may trap foreign bodies thereby making it difficult to remove the oocysts from the surface of the berry [18].

This survey focuses on *Cyclospora* and *Cryptosporidium* in fresh herbs, berries, mushrooms, and green onions. The objective is to determine the potential level of contamination of these commodities with *Cyclospora* and *Cryptosporidium*.

2.2 Sample Collection

All samples were collected from national chain and local/regional grocery stores, other conventional retail and natural food stores located across Canada. Eleven sample collection sites representing geographic regions across Canada were selected. The number of samples collected in the various regions was based on the relative proportion of the population in the respective regions. Samples were collected between May 2011 and March 2013.

2.3 Sample Distribution

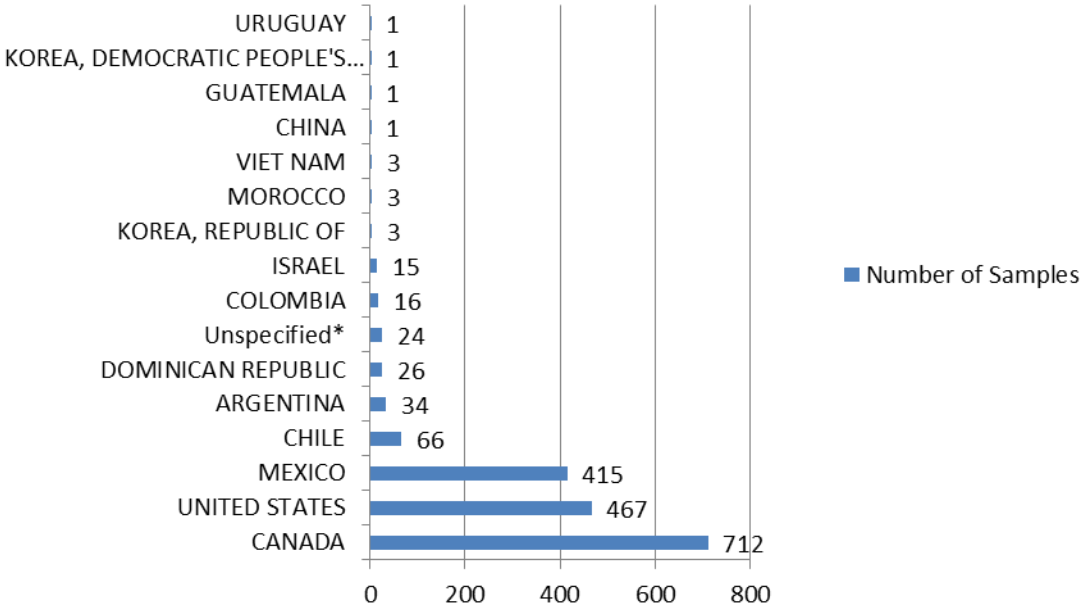
A total of 1788 samples were analyzed for the presence of *Cryptosporidium* and 1590 samples were analyzed for *Cyclospora*. A summary of the distribution by product type can be found in Table 1. The majority of the samples were from Canada, followed by the United States and Mexico as seen in Figure 1 and Figure 2. Twenty-four samples were of unknown origin.

Table 1. Distribution of Collected Samples based on Product Origin

Product Type	Product Origin			*Total*
	Imported	**Domestic**	**Unknown**	
Fresh Herbs				
Basil	32	4	2	38
Bay Leaves	1	0	0	1
Chives	8	8	1	17
Cilantro	85	35	1	121
Dill	35	21	1	57
Fenugreek	0	2	0	2
Marjoram	1	0	0	1
Mint	19	8	2	29
Mixed Herb	0	1	0	1
Oregano	8	4	0	12
Parsley	182	89	5	276
Rosemary	17	1	2	20
Sage	10	0	0	10
Savory	2	0	0	2
Sorrel	1	0	0	1
Tarragon	1	0	0	1
Thyme	5	8	0	13
Sub-Total	*407*	*181*	*14*	*602*
Berries				
Blackberries	106	4	0	110
Blueberries	148	104	0	252
Cranberries	1	12	0	13
Raspberries	59	11	0	70
Strawberries	102	40	0	142
Sub-Total	*416*	*171*	*0*	*587*
Other Fresh Produce				
Green Onion*	219	172	10	401
Mushrooms**	10	188	0	198
Sub-Total	*229*	*360*	*10*	*599*
Grand Total	**1052**	**712**	**24**	**1788**

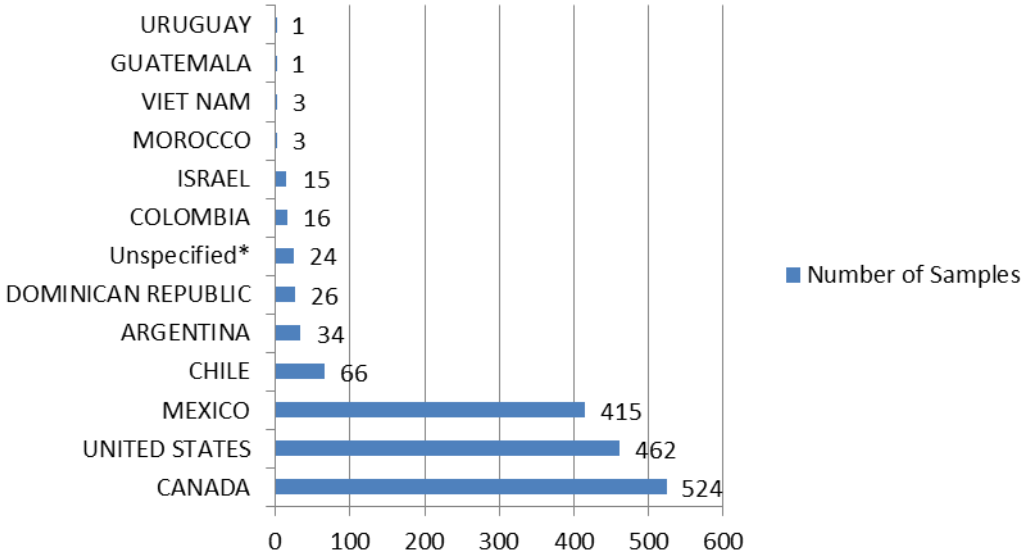
* Green onions were sampled in 2012/2013.
** Mushrooms, including white, brown, crimini, portabella, chanterelle, oyster, enoki, and shiitake varieties, were sampled in 2011/2012, and analyzed for *Cryptosporidium* spp. only.

Figure 1. Distribution of Samples Analyzed for *Cryptosporidium* spp. based on the Country of Origin (arranged by increasing number of samples)



*Unspecified refers to those samples for which the country of origin could not be determined from the product label

Figure 2. Distribution of Samples Analyzed for *Cyclospora cayetanensis* based on the Country of Origin (arranged by increasing number of samples)



*Unspecified refers to those samples for which the country of origin could not be determined from the product label

2.4 Method Details

A method based on the principles of Cook et al. (2006) was used to wash the surface of herb, berry, green onion and mushroom samples to isolate *Cyclospora cayetanensis* and/or *Cryptosporidium* spp. oocysts [19]. Oocysts, if present, were washed from the samples by stomaching (for herbs) or shaking (for berries, green onions and mushrooms) with wash buffer which was then concentrated by centrifugation and subjected to a flotation with sucrose to isolate oocysts from produce debris. DNA was extracted from the isolated oocysts and subjected to a real time polymerase chain reaction (qPCR) assay with melting curve analysis which was developed specifically by the CFIA to simultaneously detect *Cyclospora cayetanensis* and *Cryptosporidium* spp. [20, 21]. To be considered a positive result, the melting curve temperature must be a match to either the *Cyclospora* or *Cryptosporidium* control and the qPCR amplicon must be confirmed by sequencing to be *C. cayetanensis* or *Cryptosporidium* spp. DNA using a BLAST search in GenBank.

This qPCR-based method detects DNA from the target parasites and thus cannot currently discriminate viable and potentially infectious oocysts from non-viable oocysts. Therefore, detection of *C. cayetanensis* or *Cryptosporidium* spp. in a food does not necessarily mean that the microorganism in the contaminated food is capable of infection [20, 21].

2.5 Limitations

Currently, there are no internationally recognized assessment criteria for parasites in fresh produce. The methods used for detecting *C. cayetanensis* and *Cryptosporidium* spp. in produce are molecular-based methods, which do not differentiate viable (i.e., infectious) from non-viable oocysts. This means that even though a food is found to be positive for one of these parasites, the parasite is not necessarily capable of causing illness. It is therefore difficult to determine the immediate health significance of a positive result without supporting epidemiological evidence linking the food to clinical cases. Furthermore, due to the perishable nature of fresh produce, the samples tested have usually well passed their shelf-life by the time the analysis is completed, preventing the possibility of any immediate follow-up activities.

This survey was designed to elucidate the prevalence of *Cyclospora* and *Cryptosporidium* in fresh produce including herbs, berries, mushrooms and green onions available at retail. 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. There are an insufficient number of samples in this report to carry out a detailed analysis of the results based on country of origin. In cases of positive results, unsatisfactory rates between countries are not considered to be statistically comparable.

3 Results

A total of 1788 samples of fresh produce including herbs, berries, mushrooms and green onions were collected and analyzed for the presence of *Cryptosporidium*. In total, 0.4% of the samples (i.e., 8 samples including 6 samples of green onion, 1 sample of parsley and 1 sample of mushroom) were positive for the presence of *Cryptosporidium* (Table 2). Of the 1590 samples analyzed for the presence of *Cyclospora*, none were positive for the parasite (Table 3). While current molecular-based methodology can detect the presence of *Cyclospora* and *Cryptosporidium* DNA, it cannot determine if the parasite in a positive sample is viable and can infect a host.

Table 2. Summary of Results for Produce Samples Analyzed for *Cryptosporidium*

Product Type	**Product Origin**	*Cryptosporidium* spp.		
		Number of Samples	**Not Detected in 25g**	**Detected in 25g**
Berries	Domestic	171	171	-
	Imported	416	416	-
	Sub-Total	*587*	*587*	-
Green Onions	Domestic	172	170	2
	Imported	229	225	4
	Sub-Total	*401*	*395*	*6*
Herbs	Domestic	181	181	-
	Imported	421	420	1
	Sub-Total	*602*	*601*	*1*
Mushrooms	Domestic	188	187	1
	Imported	10	10	-
	Sub-Total	*198*	*197*	*1*
Grand Total		**1788**	**1780**	**8**

Table 3. Summary of Results for Produce Samples Analyzed for *Cyclospora*

Product Type	Product Origin	*Cyclospora cayetanensis*		
		Number of Samples	**Not Detected in 25g**	**Detected in 25g**
Berries	Domestic	171	171	-
	Imported	416	416	-
	Sub-Total	*587*	*587*	-
Green Onions	Domestic	172	172	-
	Imported	229	229	-
	Sub-Total	*401*	*401*	-
Herbs	Domestic	181	181	-
	Imported	421	421	-
	Sub-Total	*602*	*602*	-
Grand Total		**1590**	**1590**	**-**

4 Conclusion

Fresh produce including herbs, berries, mushrooms and green onions were collected and analyzed for the presence of *Cyclospora* and/or *Cryptosporidium.* Of the 1788 samples analyzed for *Cryptosporidium*, 0.4% (i.e., 8 samples including 6 samples of green onion, 1 sample of parsley and 1 sample of mushroom) tested positive. None of the 1590 samples analyzed for the presence of *Cyclospora* were positive for the parasite.

Positive results are followed up by the CFIA. In this case, because of the perishable nature of the products and the time elapsed between sample pick up and the completion of analysis, no fresh product was left on the market by the time the results were confirmed. Hence, no direct follow up was possible for the lots associated to the positive products. No illnesses associated with the consumption of the positive products were reported. This information was used to inform CFIA's programs and inspection activities.

Future surveys are being developed by the CFIA to better estimate the prevalence of *Cyclospora*, *Cryptosporidium* and related parasites in foods available in the Canadian market.

While the food industry and retail sectors in Canada are ultimately responsible for the food they produce and sell, and individual consumers are responsible for the safe handling of the food they have in their possession, the CFIA regulates the industry, provides oversight and promotes safe handling of foods throughout the food production chain. Surveillance activities will continue and the CFIA will inform stakeholders of its findings.

5 Acknowledgment

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

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7 Appendix A: List of Acronyms

CFIA: Canadian Food Inspection Agency

FAO: Food and Agriculture Organization of the United Nations

FDA: *Food and Drugs Act*

FDR: *Food and Drug Regulations*

FCSAP: Food and Consumer Safety Action Plan

FSAP: Food Safety Action Plan

FSSC: Food Safety Science Committee

GI: Gastro-intestinal

GAPs: Good Agricultural Practices

GMPs: Good Manufacturing Practices

PCR: Polymerase Chain Reaction

WHO: World Health Organization

g: gram

spp: species

8 Appendix B: Global Foodborne Disease Outbreaks Associated with Produce Contaminated with *Cyclospora* and *Cryptosporidium* (1995 – March 2013)

Cases of *Cyclospora cayetanensis* in Herbs						
Year	**No. Cases**	**Vehicle**	**Country**	**Province/ State**	**Source**	**History**
1997	341	Basil	United States	Multiple	CDC 1997	
1997	48	Basil	United States	Virginia	MMWR 1997, 46(30): 689-691	Basil-pesto pasta salad
1999	66	Basil	United States	Missouri	Clin Infect Dis 2001 32(7):1010-7	Chicken pasta salad associated with illness and leftover salad was positive for *Cyclopsora spp.*
2001	17	Basil	Canada	BC	Epidemiol. Infect. 2005, 133:23-27	The basil imported via the US
2004	57	Basil	United States	Illinois	FDA	Raw basil and mesculin/spring salad mix
2004	38	Basil	United States	Texas	FDA	Raw basil and mesculin/spring salad mix
2005	44	Basil	Canada	ON	Annual Report 2005	
2005	200	Basil	Canada	QC	Annual Report 2005	Pesto and pasta products made from basil from Mexico
2005	4	Basil	Canada	ON	Annual Report 2005	
2005	592	Basil	United States	Florida	CDC 2005	
2010	206	Basil	Canada	ON	Lambton Health Unit	Suspect food was cool pesto crunch.
2011	12	Cilantro and Onions	United States	Florida	CDC linelist 2011	cilantro and onions
Total	**1625**					

Cases of *Cyclospora* in Berries						
Year	**No. Cases**	**Vehicle**	**Country**	**Province/ State**	**Source**	**History**
1995	87	Raspberries	United States	Florida	Am J Trop Med Hyg 1998;59(2):235-242	January, Florida
1995	32	Raspberries	United States	New York	CDC linelist	May, New York
1995	38	Raspberries	United States	Florida	CDC linelist	August, Florida
1996	55	Berries	United States		Arch Intern Med 1998 May 25;158:1121-5	By univariate analysis illness associated with dessert containing raspberries, strawberries, blackberries, & blueberries.
1996	192	Raspberries	Canada		PHERO 2000 Vol 11 Issue 7	Outbreak number includes cases from 3 separate events/outbreaks
1996	1273	Raspberries	United States		N Engl J Med 1997 336(22):1548-56	American cases associated with outbreak in Canada from Guatemalan raspberries
1997	534	Raspberries	United States		MMWR 1997 46(23):521-23	Outbreaks in California, Florida, Maryland, Nebraska, Nevada, New York, Rhode Island, & Texas. Related to Canadian outbreak – cases reported separately. In addition, cases reported among persons on a cruise ship that departed from Florida.
1998	192	Raspberries	Canada		Can Commun Dis Rep. 1998 24(19):153-6)	13 clusters - fresh raspberries included in mixtures of berries at 12 events. Guatemala was the only source of the raspberries served at the events.

1999	94	Berries	United States		CDC linelist	Blackberries, raspberries, strawberries
1999	104	Blackberries	Canada		FDA: Outbreaks Associated with Fresh and Fresh-Cut Produce. Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce	Infected harvester
2000	19	Berries	United States		CDC linelist	Raspberries, blackberries
2000	54	Raspberries	United States		Emerg Infect Dis 2002, 8(8):783-8	Cake with cream filling & pieces of raspberries associated with illness. PCR confirmed Cyclospora DNA in filling. Raspberries from 1 Guatemalan farm & 1 Mexican farm identified as sources of raspberries.
2002	26	Raspberries	United States		CDC linelist	
2008	59	Berries	United States		CDC line list 2008	Mixed berries
2008	3	Berries	United States		CDC line list 2008	Berries
2009	8	Raspberries	United States		CDC linelist 2009	Blackberries and raspberries
Total	**2770**					
Cases of *Cryptosporidium* spp. in Produce						
Year	**No. Cases**	**Vehicle**	**Country**	**Province/ State**	**Source**	**History**
1997	54	Green onions	United States		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	

2008	16	Parsley	Sweden		Eurosurveillance, Volume 13, Issue 51	Béarnaise sauce contained chopped fresh parsley which was added after heating
2008	72	Salad	Finland		Eurosurveillance, Volume 14, Issue 28	
2010	27	Fresh herbs	Sweden		Eurosurveillance, Volume 17, Issue 46	Fresh herbs suspected. Using sequence analysis of the GP60 glycoprotein gene, a polymorphic marker with high intra-species diversity, identified the same C. parvum subtype IIdA24G1 in samples from both the Umeå outbreak and the Stockholm area cases, thus indicating a possible outbreak in the Stockholm area and establishing a link between these two events. For the outbreak in Örebro, another subtype was identified: C. parvum IIdA20G1e.
Total	**169**					

Taken from information prepared by Judy D. Greig, Public Health Agency of Canada