Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Safety Action Plan

REPORT

2010-2011 Targeted Surveys

Targeted Survey Investigating *Cyclospora cayentanensis* in Imported Fresh Herbs and Berries

RDIMS # 3755197

Canada

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.

Cyclopsora cayentanensis is a protozoan parasite which infects humans, primarily through contaminated food and water and is endemic in a number of subtropical and tropical countries. *C. cayentanensis* infection can cause mild to severe gastrointestinal (GI) symptoms (cyclosporosis) including, but not limited to, diarrhoea, weight loss, cramping, flatulence, nausea, fatigue and low grade fever.

C. cayentanensis was ranked 13th out of 24 parasites in overall global ranking for its 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). Fresh herbs and berries have been identified in the past as sources of *C. cayentanensis* contamination in Canada. This survey focussed only on imported fresh herbs and berries since Canada imports many of these commodity types from tropical and subtropical countries.

The objective of this survey was to determine the occurrence and distribution of *C. cayentanensis* contamination in imported fresh herbs and berries. A total of 513 samples of imported fresh herbs and 553 samples of imported fresh berries were collected at retail from various regions across Canada between May 2010 and March 2011. Samples included basil, chives, cilantro, mint, oregano, parsley, rosemary and thyme, as well as, blackberries, blueberries, cranberries, raspberries and strawberries.

One parsley sample from the United States was positive for *C. cayentanensis*, however, the analytical method used to detect the parasite in the sample cannot determine if the parasite is viable and potentially infectious. 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 for this lot. This information was used to inform CFIA's programs and inspection activities. It is important to note that there were no reported illnesses associated with the consumption of the product found to be positive for *C. cayentanensis*.

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 and

preparation of the food in their possession. The CFIA as well as other jurisdictions such as provincial and municipal regulators, monitor various controls throughout the food production chains. Moreover, general advice for the consumer on the safe handling and preparation 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 [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 will be collected over five 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 targeted survey focuses on *C. cayentanensis* in 1066 samples of imported berries and fresh herbs collected and analyzed between May 2010 and March 2011. This survey was designed to gather baseline information on the occurrence of *C. cayentanensis* on imported berries and fresh herbs 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 GAPs (Good Agricultural Practices) and GMPs (Good Manufacturing Practices) 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 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 food safety investigations, 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 Imported Berries and Fresh Herbs

2.1 Rationale

Cyclospora cayentanensis is a food- and waterborne parasite that is endemic in a number of subtropical and tropical regions of the world [10]. Human infection occurs through the ingestion of fresh produce or water, contaminated with human faeces that contain *C. cayentanensis* oocysts. The oocyst is a structure that can survive long periods of time outside the host, when excreted by an infected individual. The freshly excreted oocysts of *C. cayentanensis* cannot immediately infect another individual. The oocysts need specific environmental conditions, including warm temperatures, to undergo sporulation and become infective. Once sporulated and ingested by a person, the infective 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 [11] [12].

Infection with *C. cayentanensis* 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 [12]. Trade of fresh produce with countries having high rates of cyclosporosis has increased the potential that Canadians may be exposed to *C. cayentanensis* [13].

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, *C. cayentanensis* was ranked 13th out of 24 parasites in overall global public health importance [14].

Herbs and berries have been reported to be responsible for numerous outbreaks of foodborne illness. Following a joint FAO/WHO expert meeting concerning microbial hazards in leafy greens, 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 1 priority and berries a level 2 priority [15]. From 1995 to 2010, there were 27 documented outbreaks in North America associated with herbs (and products made with herbs) and berries contaminated with *C. cayentanensis* (Appendix B). 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 [13].

Fresh produce may become contaminated with *C. cayentanensis* in the field as a result of contaminated water being used for irrigation or for mixing fertilizers. *C. cayentanensis* may also be present in water used for washing produce or during the processing and packaging of the product [12]. Contaminated fingers of workers picking berries may also be a source of the parasite. Produce, such as raspberries and fresh herbs, have irregular and uneven surfaces which may trap *C. cayentanensis*. 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 *C. cayentanensis* from the surface of the berry [16].

This survey focuses on *C. cayentanensis* on imported fresh herbs and imported berries. The objective is to determine the potential level of contamination of imported fresh herbs and berries with *C. cayentanensis*.

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 2010 and March 2011.

2.3 Sample Distribution

A total of 1066 imported fresh herb (513) and imported berry (553) samples were collected between May 2010 and March 2011. A summary of the distribution can be found in Table 1a for fresh herbs and Table 1b for berries. Seventeen samples were from unknown origin. The majority of the berry samples were imported from the United States (55.2%); followed by Mexico (24.6%) and Chile (14.6%). The majority of the herbs were imported from United States (49.1%); followed by Dominican Republic (15.6%) and Colombia (14.6%).

Table 1a. Distribution of Imported Fresh Herbs by Product					
Sample Description	**Country of Origin**	**Total**	**Sample Description**	**Country of Origin**	**Total**
Basil	Colombia	13	Chives	China	1
	Denmark	1		Colombia	8
	Dominican Republic	22		Dominican Republic	8
	Israel	1		Israel	8
	Mexico	7		United States	4
	United States	8		Unknown	1
	Unknown	4	Mint	Colombia	19
	Viet Nam	1		Dominican Republic	6
Cilantro	Colombia	1		Israel	1
	Dominican Republic	4		Mexico	4
	Mexico	22		United States	7
	United States	67		Unknown	5
	Unknown	2		Viet Nam	6
Oregano	Colombia	7	Parsley	Dominican Republic	2
	Dominican Republic	14		Mexico	31
	Mexico	1		United States	158
	United States	2		Unknown	3
Rosemary	Colombia	18	Thyme	Chile	1
	Dominica	1		Colombia	9
	Dominican Republic	13		Dominica	1
	Israel	1		Dominican Republic	11
	United States	2		Israel	1
	Unknown	1		United States	4
				Unknown	1
Grand Total			**513**		

Table 1b. Distribution of Imported Berries by Product		
Sample Description	**Country of Origin**	**Total**
Blackberries	Guatemala	3
	Mexico	95
	United States	33
Blueberries	Argentina	26
	Chile	80
	Mexico	1
	United States	38
	Uruguay	1
Cranberries	United States	5
Raspberries	Chile	1
	Mexico	30
	United States	94
Strawberries	Mexico	10
	New Zealand	1
	United States	135
Grand Total		**553**

2.4 Method Details

A method based on the principles of Cook et al. (2006) was used to wash the surface of samples for analysis to detect and identify oocysts [17]. Oocysts, if present, were washed from the herb or berry samples by stomaching or shaking (respectively) 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 Polymerase Chain Reaction (PCR) assay developed specifically for this purpose by the CFIA [18]. In addition to the conventional PCR analysis, extracted DNA was also subjected to a semi-nested PCR. To be considered a positive result, a positive PCR result must be obtained for both the semi-nested and conventional PCR assays, and then be confirmed by sequencing of both amplicons as a match to *C. cayentanensis* DNA using a BLAST search in GenBank.

This PCR-based method cannot currently discriminate viable and potentially infectious oocysts, from non-viable oocysts. Therefore, detection of *C. cayentanensis* in a food does not necessarily mean that the contaminated food is capable of infection [19] [20].

2.5 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 limits 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 extension of the laboratory result to the whole production lot as it is not statistically representative. This imposes certain limitations in the generalisation of the result to the specific lot in the absence of additional information.

The survey was designed to elucidate the prevalence of *C. cayentanensis* in imported berries and fresh herbs 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 1066 samples of imported berries and fresh herbs were collected and analyzed for the presence of *C. cayentanensis.* All 553 berry samples tested, were negative for the presence of *C. cayentanensis.* Of the 513 samples of herbs analyzed, one sample, parsley from the United States, was positive for *C. cayentanensis*. While current molecular based methodology can detect the presence of *C. cayentanensis* DNA, it cannot determine if the parasite in a positive sample is viable and can infect a host. Also, due to the perishable nature of the product and the time elapsed between sample pick up and the completion of analysis no fresh product was left on the market. Hence,no direct follow up was possible for this lot. No illnesses associated with the consumption of the positive product were reported. This information was shared with the CFIA's Fresh Fruit and Vegetables program to inform CFIA's inspection activities.

Table 2 Results of Analysis for *C. cayentanensis* in Herbs and Berries

Sample Description	**Number of negative**	**Number of Positive**
Basil	57	0
Chives	30	0
Cilantro	96	0
Mint	48	0
Oregano	24	0
Parsley	193	1
Rosemary	36	0
Thyme	28	0
Subtotal	*512*	*1*
Blackberries	131	0
Blueberries	146	0
Cranberries	5	0
Raspberries	125	0
Strawberries	146	0
Subtotal	*553*	*0*
Grand Total	1065	1

4 Conclusion

A total of 1066 samples of imported fresh herbs and berries were collected from retail across Canada and tested for the presence of the parasite *C. cayentanensis.* Only one sample of fresh parsley was positive for the parasite that may cause cyclosporosis. This survey was limited in the number of samples analyzed, however, it met the objective of gathering information on the occurrence of *C. cayentanensis* on imported berries and fresh herbs.

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 the fresh product was no longer available on the market when the parasite was detected. As such, no direct follow up was possible for this lot. This information was shared with the CFIA's Fresh Fruit and Vegetables program to inform CFIA's inspection activities. It is important to note that there were no reported illnesses associated with the consumption of the product found to be positive for C. cayentanensis.

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

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).

6 References

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7 Appendix A: List of Acronyms

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
FSSC: Food Safety Science Committee
GI: Gastro-intestinal
GAPs: Good Agricultural Practices
GMPs: Good Manufacturing Practices
HC: Health Canada
PCR: Polymerase Chain Reaction
WHO: World Health Organization
°C: Degree Celsius
g: gram

8 Appendix B: Global Foodborne Disease Outbreaks Associated with Herbs and Berries Contaminated with *Cyclospora* (1995-2010)

Cases of *Cyclospora* in Basil and basil based products						
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.
Total	**1613**					
Cases of *Cyclospora* in undefined berries						
Year	**No. Cases**	**Vehicle**	**Country**	**Province/ State**	**Source**	**History**

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.
1999	94	Berries	United States		CDC linelist	Blackberries, raspberries, strawberries
2000	19	Berries	United States		CDC linelist	Raspberries, blackberries
2008	59	Berries	United States		CDC line list 2008	Mixed berries
2008	3	Berries	United States		CDC line list 2008	Berries
Total	**230**					

Cases of *Cyclospora* in Blackberries

Year	No. Cases	Vehicle	Country	Province/ State	Source	History
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
Total	**104**					

Cases of *Cyclospora* in Raspberries

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	1273	Raspberries	United States		N Engl J Med 1997 336(22):1548-56	American cases associated with outbreak in Canada from Guatemalan raspberries
1996	192	Raspberries	Canada		PHERO 2000 Vol 11 Issue 7	Outbreak number includes cases from 3 separate events/outbreaks

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.
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	
2009	8	Raspberries	United States		CDC linelist 2009	Blackberries and raspberries
Total	**2436**					

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