Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Targeted Survey

REPORT

2013/14 Targeted Surveys

Targeted Surveys Investigating *Cyclospora cayetanensis* and *Cryptosporidium* spp. in Fresh Leafy Herbs and Green Onions

RDIMS # 6624751

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 health risk. The information gained from these surveys provides both support for the prioritization of the Agency's activities to areas of greater concern, and scientific evidence to address areas of lesser concern. Originally started under the Food Safety Action Plan (FSAP), targeted surveys have been incorporated into the CFIA's regular surveillance activities as a valuable tool for generating information on certain hazards in foods, identifying/characterizing new and emerging hazards, informing trend analysis, prompting/refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

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 green onions have been identified in the past as sources of *Cyclospora* and *Cryptosporidium* contamination in Canada. This survey focussed on fresh leafy herbs and green onions.

The objective of this survey was to determine the occurrence and distribution of *Cyclospora* and *Cryptosporidium* contamination in fresh produce such as fresh leafy herbs and green onions. A total of 1116 samples were analyzed for the presence of *Cyclospora* and *Cryptosporidium*. Samples were collected at retail from various regions across Canada between April 2013 and March 2014. None of the samples were positive for either *Cyclospora* or *Cryptosporidium*.

The Canadian Food Inspection Agency 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 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 Targeted Surveys

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

Targeted surveys are used to gather information regarding the possible occurrence or prevalence of hazards in defined food commodities. These surveys generate essential information on certain 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 as appropriate.

Due to the vast number of hazard and food commodity combinations, it is not possible, nor should it be necessary, to use targeted surveys to identify and quantify all hazards in foods. 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.

This survey was designed to gather baseline information on the occurrence of *Cyclospora* and *Cryptosporidium* on fresh leafy herbs and green onions available to Canadians at retail between April 2013 and March 2014.

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. 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) [1] and the *Recommended International Code of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969) [1]. 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 the 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) [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.

Fresh fruits and vegetables that are imported into Canada or domestically produced and marketed inter-provincially must also comply with the safety requirements of the *Fresh Fruit and Vegetable Regulations* [5] under the *Canada Agricultural Products Act* [6]. 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.

The 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 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 Leafy Herbs and Green Onions

2.1 Rationale

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, fresh herbs were deemed as a level one priority while green onions were deemed as a level two priority [7]. From 1995 to 2014, there were fourteen documented outbreaks in North America associated with herbs (and products made with herbs) or green onions contaminated with *Cyclospora* (Appendix B) and one

outbreak associated with green onions contaminated with *Cryptosporidium*. Leafy herbs and green onions were identified as being in the top six produce commodities attributed to increased produce-associated foodborne disease outbreaks in the U.S. from 1996 to 2008 [8].

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 [9]. 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 fresh herbs.

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 [9]. Trade of fresh produce with countries having high rates of cyclosporosis (*Cyclospora* infection) has increased the potential that Canadians may be exposed to *Cyclospora* [10]. *Cryptosporidium* is prevalent worldwide and was reported in 2004 to have infected approximately 4% of the North American population [11].

The objective of this survey is to determine the potential level of contamination of fresh leafy herbs and green onions with *Cyclospora* and *Cryptosporidium*.

2.2 Targeted Parasites

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 [12], *Cryptosporidium* can be found in both developed and developing countries worldwide with varying levels of prevalence [11,13]. 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, *Cryptosporidium* was ranked 5th and *Cyclospora* was ranked 13th out of 24 parasites in overall global public health importance [14].

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 [9]. 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.

2.3 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 April 2013 and March 2014.

2.4 Method Details

A method based on the principles of Cook *et al.* (2006) was used to wash the surface of leafy herb or green onion samples to isolate *Cryptosporidium* spp. and *Cyclospora cayetanensis* oocysts [16]. Oocysts, if present, were washed from the samples by stomaching (for leafy herbs such as cilantro, basil, parsley, mint, tarragon, savoury, dill, sorrel and marjoram) or shaking (for thyme, sage, chives and oregano) with a glycine wash buffer, or shaking with a sodium pyrophosphate wash buffer (for green onions). The produce washes were 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 by the CFIA specifically to simultaneously detect *Cyclospora cayetanensis* and *Cryptosporidium* spp. [17,18] 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. DNA in a food sample does not necessarily mean that consumption of the implicated food will result in illness.

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 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 leafy herbs 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

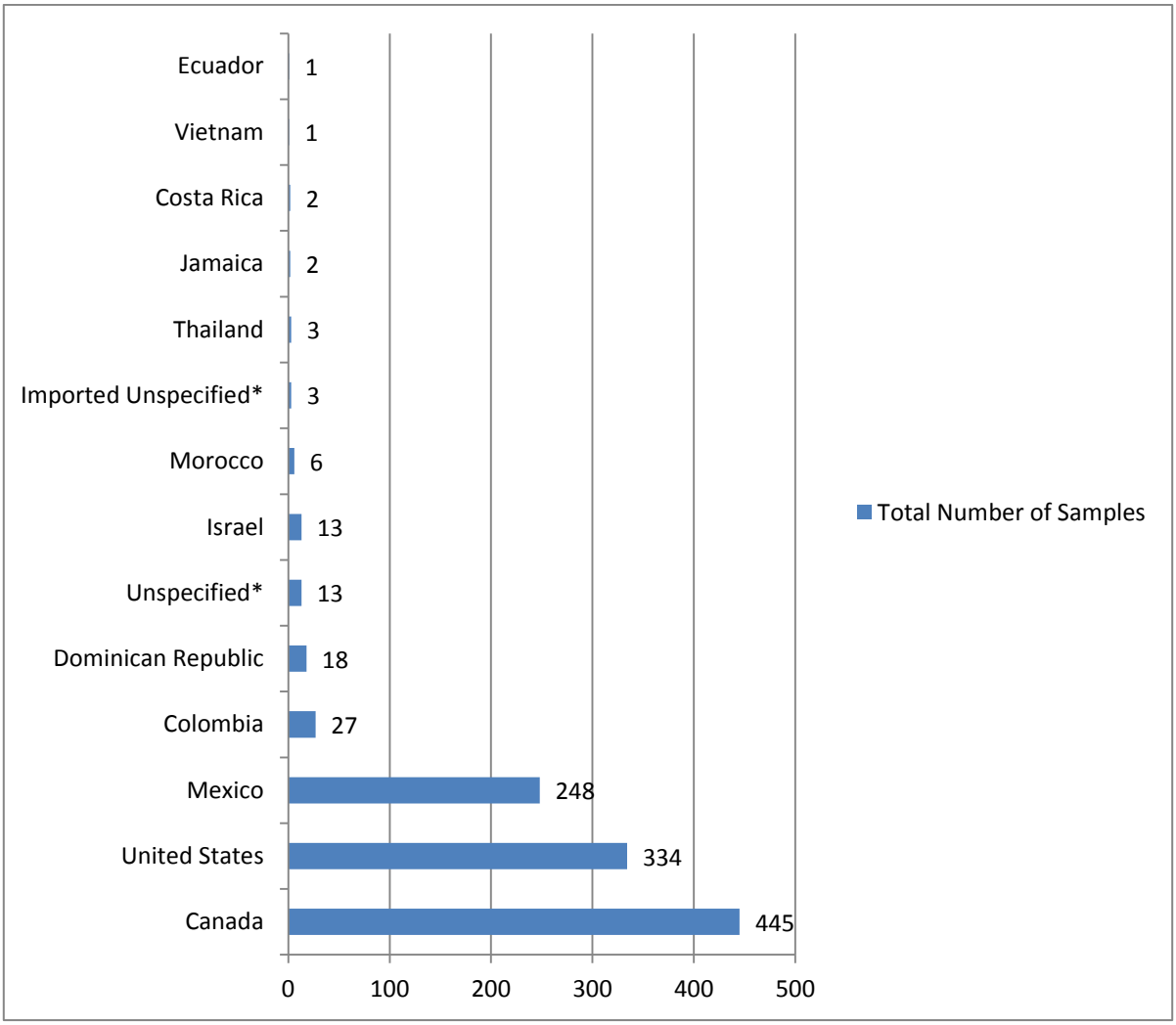
3.1 Sample Distribution

A total of 1116 samples were analyzed for the presence of *Cryptosporidium* and *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. The country of origin was not specified on 13 samples and 3 samples were known to be imported but the country of origin was not specified.

Table 1 Distribution of Collected Samples by Product Type based on Product Origin

Product Type	Product Origin			Total
	Imported	Domestic	Unknown	
Fresh Leafy Herbs	*473*	*274*	*4*	***751***
Basil	26	12	0	**38**
Chives	9	9	0	**18**
Cilantro	89	62	0	**151**
Coriander	4	3	0	**7**
Dill	61	46	1	**108**
Lemon Basil	1	0	0	**1**
Marjoram	4	0	0	**4**
Mint	25	17	1	**43**
Mojito Mint	1	0	0	**1**
Oregano	13	0	0	**13**
Parsley	202	116	2	**320**
Sage	9	4	0	**13**
Savory	8	3	0	**11**
Sorrel	2	0	0	**2**
Tarragon	9	0	0	**9**
Thyme	10	2	0	**12**
Green Onions	*185*	*171*	*9*	***365***
Grand Total	**658**	**445**	**13**	**1116**

Figure 1 Distribution of Samples Analyzed for *Cryptosporidium* spp. and 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

3.2 Summary of Results

A total of 1116 samples of fresh leafy herbs and green onions were collected and analyzed for the presence of *Cryptosporidium* and for the presence of *Cyclospora.* None of the samples tested positive for either parasite (Table 2) so no follow-up activities by the CFIA were required.

Table 2 Summary of Results for Produce Samples Analyzed for *Cyclospora cayetanensis* and *Cryptosporidium* spp.

Product Type	Product Origin	*Cyclospora cayetanensis* and *Cryptosporidium* spp.		
		Number of Samples	**Not Detected in 25g**	**Detected in 25g**
Leafy Herbs	Domestic	274	274	0
	Imported	473	473	0
	Unknown	4	4	0
	Sub-Total	*751*	*751*	*0*
Green Onions	Domestic	171	171	0
	Imported	185	185	0
	Unknown	9	9	0
	Sub-Total	*365*	*365*	*0*
Grand Total		**1116**	**1116**	**0**

4 Conclusion

A total of 1116 samples of fresh leafy herbs and green onions were collected and analyzed for the presence of *Cyclospora* and *Cryptosporidium.* No results requiring follow-up activities by the CFIA were obtained in this survey since none of the samples tested positive for the presence of *Cryptosporidium* or *Cyclospora*.

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).

6 References

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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

qPCR: Real-Time Polymerase Chain Reaction

WHO: World Health Organization

g: gram

spp: species

Appendix B: Global Foodborne Disease Outbreaks Associated with Produce Contaminated with *Cyclospora* and *Cryptosporidium* (1995-2014)

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	
2008	16	Fresh Parsley	Sweden		Eurosurveillance, Vol 13, Issue 51, 2008	Béarnaise sauce contained chopped fresh parsley which was added after heating.
2010	206	Basil	Canada	ON	Lambton Health Unit	Suspect food was cool pesto crunch.
2011	12	Cilantro and Onions	United States	Florida	CDC linelist	

2014	304	Cilantro	United States	Multiple	CDC	Epidemiologic and traceback investigations conducted in Texas by state and local public health and regulatory officials and the FDA indicated that some illnesses among Texas residents were linked to fresh cilantro from Puebla, Mexico. There is no evidence to suggest that any illnesses outside of Texas are linked to cilantro from Puebla, Mexico. Reported cases of cyclosporiasis in Texas have returned to baseline levels in August; therefore, it is likely that the outbreak has ended.
2014	207	Cilantro	United States	Multiple (mostly Texas)	CDC	7 hospitalized- Most (133; 64%) of the cases were reported from Texas. Traceback investigations conducted in Texas indicated that some illnesses among Texas residents were linked to fresh cilantro from Puebla, Mexico.
Total	**2152**					

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	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	**153**					

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