Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Food Microbiology – Targeted Surveys

FINAL REPORT

Parasites in Fresh Leafy Vegetables

April 1, 2014 – March 31, 2016

Canada

Summary

Produce such as berries, herbs and vegetables have been identified in the past as sources of contamination for parasites. Produce can become contaminated with parasites during production, harvest, post-harvest handling, processing, packaging and distribution. Parasites can infect humans primarily through contaminated food and water. In general, symptoms of infection can include mild to severe flu-like (*Toxoplasma*) and gastrointestinal symptoms (*Cyclospora*, *Cryptosporidium*, *Giardia*). Previous targeted surveys have reported on the occurrence and distribution of *Cyclospora cayetanensis* (*C. cayetanensis)* and *Cryptosporidium spp*. in berries, fresh leafy herbs, mushrooms and green onions. This report focuses the occurrence and distribution of *C. cayetanensis, Cryptosporidium spp*., *Toxoplasma gondii (T. gondii),* and *Giardia spp.* in minimally processed (pre-washed and pre-packaged) fresh leafy vegetables.

Considering the factors mentioned above and their relevance to Canadians, fresh leafy vegetables were selected for targeted surveys. Over the course of this study (April 1, 2014 to March 31, 2016), a total of 2233 samples of leafy vegetables were collected from retail locations in 11 cities across Canada and tested for parasites of concern (*C. cayetanensis, Cryptosporidium spp., T. gondii, and Giardia spp.*). Parasite DNA of *Cryptosporidium spp., and T. gondii* were detected in 0.1% (2/2233) and 0.1% (3/2233) of the leafy vegetable samples, respectively. *C.cayetanensis and Giardia spp.* DNA were detected in 0.3% (6/2233) and 0.7% (15/2233) of the leafy vegetable samples respectively.

Due to the perishable nature of the products and the time elapsed between sample pick up and the completion of analysis, the implicated products were no longer available on the market when the parasite DNA was detected and as such, no direct product action was possible. In addition, the analytical methods used to analyse the samples are unable to discriminate between infectious and non-infectious parasites, rendering it difficult to determine if these products represented an immediate health risk. There were no reported illnesses linked to the parasite positive samples.

Overall, our survey results suggest that most fresh leafy vegetables were free of parasitic contamination. Regardless, fresh leafy vegetables are a known potential source of foodborne illness causing parasites and as such, safe handling practices are recommended for producers, retailers and consumers.

What Are Targeted Surveys?

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 support for the allocation and prioritization of the Agency's activities to areas of greater concern. Originally started as a project under the Food Safety Action Plan (FSAP), targeted surveys have been embedded in the CFIA's regular surveillance activities since 2013. Targeted surveys are a valuable tool for generating information on certain hazards in foods, identifying and characterizing new and emerging hazards, informing trend analysis, prompting and refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

Food safety is a shared responsibility. The Canadian Food Inspection Agency works with federal, provincial, territorial and municipal governments and provides regulatory oversight of the food industry to promote safe handling of foods throughout the food production chain. The food industry and retail sectors in Canada are 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.

Why Did We Conduct This Survey?

Fresh leafy vegetables are frequently consumed by Canadians in all age groups[1]. Unfortunately, they have been implicated in numerous foodborne illness outbreaks worldwide[2] due to contamination by parasites. According to the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) multicriteria-based ranking for risk management of food-borne parasites, fresh produce has been categorised as the primary food vehicles of *Cyclospora cayetanensis (C. cayetanensis*) and *Cryptosporidium spp.* and secondary food vehicles of *Toxoplasma gondii* (*T. gondii)* [2]. Fresh produce can be exposed to contaminated water during primary production and postharvest processing, as well as to inadequately composted organic fertilizers (manure) during primary production[2].

Given the above, minimally processed (pre-washed, pre-packaged) fresh leafy vegetables were selected for targeted surveys over a 2 fiscal year period starting in 2014 to gather baseline information on the occurrence of parasites in this commodity available at retail in Canada. This report details results of the entire survey period (April1, 2014 to March 31, 2016).

What Did We Sample?

For this survey, a sample consisted of a single unit (e.g., individual consumer-size package(s) from a single lot) with a total weight of at least 200g. All samples were collected from national retail chains and local/regional grocery stores located in 11 major cities across Canada. These

cities encompassed four geographical areas: Atlantic (Halifax and Saint John), Quebec (Quebec City, Montreal), Ontario (Toronto, Ottawa), and the West (Vancouver, Kelowna, Calgary, Saskatoon and Winnipeg). The number of samples collected from these cities was in proportion to the relative population of the respective areas. Samples were collected between April 1, 2014 and March 31, 2016. Imported samples were collected year round with reduced numbers of samples collected in the summer months. Domestic samples were collected throughout the summer and early fall (June to October). Minimally processed pre-packaged leafy vegetables were sampled including those both conventionally and organically produced.

What Analytical Methods Were Used and How Were Samples Assessed?

Samples were analyzed using CFIA internally developed methods. The 18S rDNA qPCR, melting curve analysis, and sequencing confirmation methods were used for the detection of *C. cayetanensis*, *Cryptosporidium spp*, *T. gondii* [3]. A nested PCR method was used to detect *Giardia spp*. DNA[4, 5].

At the time of writing this report, no assessment guidelines had been established in Canada for parasites in fresh produce. In addition, the analytical methods used to analyse the samples detect the presence of parasite DNA and cannot discriminate between viable (potentially infectious) and non-viable (non-infectious) parasites. The detection of parasite DNA was therefore assessed as investigative indicating that further consideration is warranted to determine which follow-up activities would be the most appropriate. (Table 1).

Table 1 Analytical Methods and Assessment Criteria for Detection of Parasite DNA in Fresh Leafy Vegetables

Parasite DNA Analysis	Method(s)	Assessment Criteria	
		Satisfactory	Investigative
C. cayetanensis	qPCR assay, melting curve analysis, & sequencing	Not detected	Detected
Cryptosporidium spp.		Not detected	Detected
T. gondii		Not detected	Detected
Giardia spp.	Nested PCR	Not detected	Detected

What Were the Survey Results?

A total of 2233 leafy vegetable samples were analysed for *C. cayetanensis, Cryptosporidium spp., T. gondii* and *Giardia spp.* (Table 2). No parasite DNA was detected in most (98.8%) of the samples tested, while 1.2% (26/2233) were found to be positive for parasite DNA.

Table 2 Assessment Results of Fresh Leafy Vegetable Samples

Number of Samples Tested	Satisfactory Assessment	Investigative Assessment			
		C. cayetanensis	***Cryptosporidium spp.***	***T. gondii***	***Giardia spp.***
2233	2207 (98.8%)	6	2	3	15
		26 (1.2%)			

Seventy-eight percent (78%, 1733/2233) of the samples tested were conventionally produced while 22% (500/2233) were organically produced. Forty-two percent (42%) of the parasite DNA positive samples were conventionally produced and 58% were organically produced (Table 3).

Table 3 Investigative Sample Distribution by Production Practice

Production Practice	Number of Samples (% of total samples tested)	Investigative Samples				Total Number of Investigative Samples (% of positive samples)
		C. cayetanensis	***Cryptosporidium spp.***	***T. gondii***	***Giardia spp.***	
Conventional	1733 (78%)	1	1	1	8	11 (42%)
Organic	500 (22%)	5	1	2	7	15 (58%)
Total	2233 (100%)	6	2	3	15	26 (100%)

Of the 2233 samples tested, 75% (1671/2233) were imported and 25% (562/2233) were domestic. Ninety-two percent (92%, 24/26) of the parasite DNA positive samples were imported, while 8% (2/26) were domestic (Table 4).

Table 4 Investigative Sample Distribution by Product Origin

Product Origin	Number of Samples (% of total samples tested)	Investigative Samples				Total Number of Investigative Samples (% of positive samples)
		C. cayetanensis	*Cryptosporidium spp.*	*T. gondii*	*Giardia spp.*	
Domestic	562 (25%)	0	0	0	2	2 (8%)
Imported	1671 (75%)	6	2	3	13	24 (92%)
Total	2233 (100%)	6	2	3	15	26 (100%)

C. cayetanensis and *T. gondii* DNA positive samples were found in the fall. *Cryptosporidium spp.* DNA positive samples were found in the spring. *Giardia spp*. DNA positive samples were found in the winter, spring and summer (Table 5).

Table 5 Imported and Domestic Sample Distribution by Season Sampled

Sampling Season	Number of Samples Tested	*C. cayetanensis*	*Cryptosporidium spp*	*T. gondii*	*Giardia spp.*	Total Number of Investigative Samples (% of samples tested)
Spring (March - May)	496	0	2	0	1	3 (0.6%)
Summer (June - August)	562	0	0	0	3	3 (0.5%)
Fall (September - November)	635	6	0	3	0	9 (1.4%)
Winter (December - February)	540	0	0	0	11	11 (2.0%)
Total	2233	6	2	3	15	26 (1.2%)

More than 12 types of leafy vegetables were collected and analysed (Table 6). DNA of *Giardia spp.*, *C. cayetanensis, Cryptosporidium spp., and T. gondii* were detected in various types of leafy vegetables.

Table 6 Sample Distribution by Product Type

Product type	Number of Samples (% of total samples tested)	Number of Investigative Samples				Total Number of Investigative Samples / Product Type
		C. cayetanensis	*Cryptosporidium spp.*	*T. gondii*	*Giardia spp.*	
Arugula	262 (11.7%)	4	1	0	5	10
Spinach	946 (42.4%)	1	1	3	6	11
Mixed Leafy	502 (22.5%)	1	0	0	0	1
Lettuce	364 (16.3%)	0	0	0	2	2
Kale	82 (3.7%)	0	0	0	1	1
Chard	39 (1.8%)	0	0	0	1	1
Collard	14 (0.6%)	0	0	0	0	0
Dandelion	16 (0.7%)	0	0	0	0	0
Others*	8 (0.3%)	0	0	0	0	0
Total	2233 (100%)	6	2	3	15	26

*Mache, Rappini, Broccoli Leaf, Escarole (≤3 samples per type)

What Do the Survey Results Mean?

In this survey, approximately 99% (2207/2233) of the minimally processed (pre-washed and pre-packaged) fresh leafy vegetable samples analyzed were free of specific parasites. A total of 26 samples (1.2%) were found to be positive for parasite DNA. Parasite DNA of *Cryptosporidium spp.*was detected in 0.1% (2/2233), *T. gondii* in 0.1% (3/2233), *C.cayetanensis* in 0.3% (6/2233) and *Giardia spp.* in 0.7% (15/2233) of the leafy vegetable samples tested.

Previous surveillance studies have reported varied positive rates for these parasites in leafy greens. The parasite positive rates found in our study are lower than those reported in a 2007-2008 Saudi Arabian study [6] and a 2009-2010 Canadian study [7]. The 2007-2008 Saudi Arabian

study[6] of *Toxoplasma gondii* and *Giardia lamblia* in a total of 470 samples of retail leafy vegetables showed a positive rate of 1.1% and 5.1% respectively. The 2009-2010 Canadian [7] study of *Cyclospora spp., Cryptosporidium spp.* and *Giardia duodenalis* in a total of 544 ready-to-eat (RTE) fresh-cut leafy samples reported positive rates of 1.7%, 5.9% and 1.8% respectively. The differences in the positive rates of parasites in leafy vegetables between these studies may be attributable to differences in product type (pre-washed vs. non pre-washed), detection methodology (DNA-based vs. microscopy), as well as agricultural practices[7]. See Table 7 for a comparison of the parasite prevalence rates from various studies.

Table 7 Comparison of Prevalence of Parasite DNA in Various Studies

C. cayetanensis	*Cryptosporidium spp.*	*T. gondii*	*Giardia spp.*	Total Number of Samples	Reference
0.3%	0.1%	0.1%	0.7%	2233	Present study
1.7%	5.9% (5.3% *C.parvum*)	Not Applicable (N/A)	1.8% (*Giardia duodenalis*)	544	2009-2010 Canadian Study
N/A	N/A	1.1%	5.1% (*Giardia lamblia*)	470	2007-2008 Saudi Arabian Study

In our survey, only *Giardia spp.* DNA was detected in domestic samples, while DNA of all four parasites were detected in imported samples (Table 4). In addition, parasite DNA was more frequently detected in imported organic samples than that in imported conventional samples. These observations made be due to the fact that *C.cayentanensis* is not endemic to Canada, differences in regional climate where the produce is grown and differences in pre and post-harvest practices.

We observed parasite DNA positive samples in all four seasons (Table 5) with the seasonality appearing to be parasite specific. The Canadian study [7] showed a distinct seasonality with 88% of the positive samples detected in samples from the summer and early fall. The Saudi Arabian study [6] found positive samples in all four seasons with significantly higher positive rates in the spring followed by the summer. The seasonal differences may be attributable to differences in

the regional climate where the produce was grown along with differences in pre and post-harvest practices.

Due to the perishable nature of the products and the time elapsed between sample pick up and the completion of analysis, the implicated products were no longer available on the market when the parasite DNA was detected and as such, no direct product action was possible. In addition, the analytical methods used to analyse the samples are unable to discriminate between infectious and non-infectious parasites, rendering it difficult to determine if these products represented an immediate health risk. There were no reported illnesses linked to the parasite positive samples.

Overall, our survey results suggest that most fresh leafy vegetables were free of parasitic contamination. Regardless, fresh leafy vegetables are a known potential source of foodborne illness causing parasites and as such, safe handling practices are recommended for producers, retailers and consumers.

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