



Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

# Food Microbiology – Targeted Surveys

## FINAL REPORT

### Parasites in Fresh Whole Mushrooms

April 1, 2016 – March 31, 2017



# Summary

Fresh produce such as berries, herbs and vegetables have been identified in the past as sources for parasites and has been implicated in numerous foodborne illness outbreaks worldwide. Fresh produce can become contaminated with parasites during production, harvest, post-harvest handling, packaging and distribution. Previous targeted surveys have reported on the occurrence of *Cryptosporidium* species (spp.) in fresh mushrooms and on the occurrence of *Cyclospora cayetanensis* (*C. cayetanensis*) and *Cryptosporidium* spp. in berries, fresh leafy herbs and green onions. This report focuses the occurrence of *C. cayetanensis*, *Cryptosporidium* spp., *Toxoplasma gondii* (*T. gondii*), and *Giardia* spp. in fresh mushrooms.

Considering the factors mentioned above and their relevance to Canadians, fresh mushrooms were selected for targeted surveys. Over the course of this study (April 1, 2016 to March 31, 2017), a total of 483 samples of fresh mushrooms 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.). All samples were found to be free of parasite deoxyribonucleic acid (DNA). Regardless, fresh produce 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?

According to the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) ranking for risk management of food-borne parasites, fresh produce has been implicated in numerous foodborne illness outbreaks worldwide<sup>1</sup>. Additionally, fresh produce has been categorised as the primary food vehicles of *Giardia duodenalis*, *Cyclospora cayetanensis* (*C. cayetanensis*) and *Cryptosporidium* species (spp.) and secondary food vehicles of *Toxoplasma gondii* (*T. gondii*)<sup>1</sup>. Fresh produce can be exposed to contaminated water during primary production and postharvest handling, as well as to inadequately composted organic fertilizers (manure) during primary production<sup>1</sup>.

A previous targeted survey, conducted from April 1, 2011 to March 31, 2012 detected the presence of *Cryptosporidium* spp. deoxyribonucleic acid (DNA) in 1 of 198 (0.5%) fresh mushroom samples analysed. The 2011-2012 targeted survey results can be found in a [separate report](#).

Given the above and the fact that fresh mushrooms are frequently consumed by Canadians in all age groups<sup>2</sup>, this commodity was selected for a one year targeted survey from April 1, 2016 to March 31, 2017 to further investigate the occurrence of a broader range of parasites (*Cryptosporidium* spp., *C. cayetanensis*, *T. gondii*, *Giardia* spp.) in this commodity available at retail in Canada.

## 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 250g. 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.

A total of 483 whole mushroom samples were collected throughout the year between April 1, 2016 and March 31, 2017. Almost all of the samples (99%, 478/483) were grown in Canada. Of the 483 samples, 72% (348/483) were conventionally produced while 28% (135/483) were organically produced.

Three types of whole mushrooms were collected and analysed: 70% (336/483) White Button; 29% (141/483) Brown Cremini; and 1% (6/483) Baby Bella.

## 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., and *T. gondii*<sup>3</sup> DNA. A nested PCR method was used to detect *Giardia* spp. DNA<sup>4,5</sup>.

At the time of writing this report, no assessment guidelines had been established in Canada for parasites in fresh produce. The analytical methods used in this survey detect the presence of parasite DNA and cannot discriminate between living and dead parasites. Therefore, the detection of parasite DNA was assessed as “investigative” and required further consideration to determine appropriate follow-up actions (Table 1).



**Table 1 - Analytical Methods and Assessment Criteria for the Detection of Parasite DNA in Fresh Whole Mushrooms**

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

## What Were the Survey Results?

A total of 483 whole mushroom samples were analysed for *C. cayetanensis*, *Cryptosporidium* spp., *T. gondii* and *Giardia* spp.. No parasite DNA was detected in any (0/483) of the samples tested.

## What Do the Survey Results Mean?

In this survey, all (100%) of the fresh whole mushroom samples analyzed were free of the four parasites tested for.

A literature search found that previous surveillance studies have been conducted on parasites in fresh produce sold at retail. Unfortunately, these previous studies did not include mushrooms. Two examples are a 2007-2008 Saudi Arabian study<sup>6</sup> and a 2014-2016 Indian study<sup>7</sup>. The 2007-2008 Saudi Arabian study<sup>6</sup> 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 2014-2016 study conducted in India<sup>7</sup> investigated the presence of *Cryptosporidium* spp. and *Giardia* spp. in a total of 284 retail fresh produce samples and found a prevalence rate of 6% and 4.5% respectively. The differences in the positive rates of parasites between studies may be attributable to differences in product type, detection methodology (DNA-based vs. microscopy), as well as agricultural practices. See Table 2 for a comparison of the parasite prevalence rates between various studies.

**Table 2 - Comparison of Parasite Prevalence in Various Studies**

Reference	Commodity	Detection Method	<i>C. cayetanensis</i>	<i>Cryptosporidium</i> spp.	<i>T. gondii</i>	<i>Giardia</i> spp.	Total Number of Samples
Current study	Whole Mushrooms	DNA-based	0%	0%	0%	0%	483
2011-2012 Targeted Survey <sup>8</sup>	Whole Mushrooms	DNA-based	N/A	0.5%	N/A	N/A	198
2007-2008 Saudi Arabian Study <sup>6</sup>	Spinach Radish Leek Parsley Green Onion Dill Lettuce Cabbage Watercress Coriander	Microscopy and DNA-based	N/A	N/A	1.1%	5.1% ( <i>Giardia lamblia</i> )	470
2014-2016 Indian Study <sup>7</sup>	Cabbage Carrot Chili Coriander Cucumber Radish Turnip Tomato	Microscopy	N/A	6% ( <i>C. parvum</i> identified in 2/17 <i>Cryptosporidium</i> spp. positive samples)	N/A	4.6% ( <i>Giardia duodenalis</i> identified in 2/13 <i>Giardia</i> spp. positive samples)	284

N/A: not applicable

Our survey results show that all samples tested were free of parasite DNA. Regardless, fresh produce are a known potential source of foodborne illness causing parasites and as such, safe handling practices are recommended for producers, retailers and consumers.

## References

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3. Lalonde, L.F. and A.A. Gajadhar, *Optimization and validation of methods for the isolation and real-time PCR identification of protozoan oocysts on leafy green vegetables and berry fruits* Food and Waterborne Parasitology, 2016. **2**(March 2016): p. 1-7.
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7. Utaaker, K.S., et al., *Checking the detail in retail: Occurrence of Cryptosporidium and Giardia on vegetables sold across different counters in Chandigarh, India*. International Journal of Food Microbiology, 2017. **263**: p. 1-8.
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