

Bacterial Pathogens in a Variety of Refrigerated, Multi-Ingredient, Ready-to-Eat Processed Foods - April 1, 2013 to March 31, 2018

Food microbiology - Targeted surveys - Final report





## **Summary**

Ready-to-eat (RTE) foods are defined as foods not requiring any further preparation before consumption except perhaps washing/rinsing, thawing or warming. RTE foods have become increasingly popular across all age groups due to their convenience. Unfortunately, some RTE foods have been associated with recalls and outbreaks of foodborne illnesses in Canada and worldwide. RTE foods can range in complexity from single ingredient (for example, chopped lettuce) to multi-ingredient (for example, guacamole) foods. As the number of ingredients increases so does the risk of cross contamination during production. As with all foods, RTE foods can also be contaminated during handling, packaging and distribution. Most importantly, given that these foods are consumed "as is," the presence of bacterial pathogens presents an increased potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, a variety of multi-ingredient RTE processed foods requiring refrigeration (dips, sauces, salad dressings, prepared salads) were selected for targeted surveys. The purpose of the surveys was to generate baseline information on the occurrence of indicator and pathogenic bacteria in a variety of these RTE foods. Over the course of this four year study (April 1, 2013 to March 31, 2014 and April 1, 2015 to March 31, 2018), a total of 4851 samples were collected from retail locations in 11 cities across Canada. Throughout the entire survey period, all samples were tested for generic *Escherichia coli* (*E.coli*) and the pathogens *Listeria monocytogenes* (*L.monocytogenes*) and *Salmonella* species (spp.). Throughout the second, third and fourth year of the study, 3282 samples were tested for the pathogens *Bacillus cereus* (*B. cereus*), *Clostridium perfringens* (*C.perfringens*) and *Staphylococcus aureus* (*S.aureus*). Generic *E.coli* is an indicator organism as the levels at which they are found in foods is used to assess the overall sanitation conditions throughout the food production chain.

*C. perfringens, Salmonella* spp, and generic *E.coli* (>  $10^2$  Most Probable Number (MPN)/gram (g)) or Colony Forming Units (CFU)/g) were not found in any samples. Presumptive *B. cereus* was detected at elevated levels ( $10^3 < x \le 10^4$  CFU/g) in 2/3282 (0.06%) samples. S. aureus was detected at elevated levels ( $10^2 < x \le 10^4$  CFU/g) in 1/3282 (0.03%) samples. *L. monocytogenes* was detected in 5/4851 (0.1%) samples, of which three were considered Category 1 and two were considered Category 2.

In RTE dips, salads and sauces, the presence of elevated levels of presumptive *B. cereus* ( $10^3 < x \le 10^4$  CFU/g) and *S. aureus* ( $10^2 < x \le 10^4$  CFU/g) indicate that the food may have been produced under unsanitary conditions. Depending on the product and levels found, samples containing *L. monocytogenes* may indicate that the food was produced under unsanitary conditions, and may be deemed unfit for human consumption.

The Canadian Food Inspection Agency (CFIA) conducted appropriate follow-up activities such as facility inspections and additional sampling. Recalls were issued in response to those Category 1, *L. monocytogenes* positive samples where the affected product was still on the market. The source of contamination was determined in one case and preventive and corrective actions were implemented by the manufacturing facilities. No reported illnesses were associated with any of the contaminated products.

Overall, our survey results suggest that almost all multi-ingredient RTE processed foods available for purchase at retail in Canada are safe for consumption. They can however be found to be contaminated with pathogens such as those found in this study: *B. cereus, S. aureus* and *L. monocytogenes*. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

## What are targeted surveys?

Targeted surveys are used by the 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 CFIA 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?

RTE foods are defined as foods not requiring any further preparation before consumption except perhaps washing/rinsing, thawing or warming. Pre-prepared RTE foods such as those selected for this survey (dips, sauces, salad dressings, prepared salads) have become increasingly popular across all age groups<sup>1</sup> due to their convenience. Unfortunately, some have been associated with recalls and outbreaks of foodborne illnesses in Canada<sup>2</sup> and worldwide<sup>3-6</sup>. RTE foods can range in complexity from single ingredient (for example, chopped lettuce) to multi-ingredient (for example, guacamole) foods. As the number of ingredients increases so

does the risk of cross contamination during production. As with all foods, RTE foods can also be contaminated during handling, packaging and distribution. Most importantly, given that these foods are consumed "as is," the presence of bacterial pathogens presents an increased potential risk for foodborne illnesses.

Considering the factors mentioned above and their relevance to Canadians, a variety of multiingredient RTE foods requiring refrigeration were selected for targeted surveys from April 1,
2013 to March 31, 2014 and April 1, 2015 to March 31, 2018. This survey was implemented to
gain further information on the occurrence of indicator (generic *E.coli*) and pathogenic (*B.*cereus, *C.* perfringens, Salmonella spp., *S.* aureus, *L.* monocytogenes) bacteria in this product
type at retail in Canada. Generic *E. coli* is an indicator organism as the levels at which they are
found in foods is used to assess the overall sanitation conditions throughout the food production
chain.

## What did we sample?

For this survey, a sample consisted of a single or multiple unit(s) (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)
- 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, 2013 to March 31, 2014 and April 1, 2015 to March 31, 2018. A variety of domestic, imported, conventional and organic RTE dips, sauces, salad dressings and prepared salads were sampled. Sample collection was evenly distributed throughout the year.

## What analytical methods were used and how were samples assessed?

Samples were analyzed using methods published in Health Canada's Compendium of Analytical Methods for the Microbiological Analysis of Foods <sup>7</sup> (table 1). The assessment criteria used in this survey (table 1) are based on international food safety authorities' microbiological guidelines<sup>8-11</sup>.

Table 1 - Analytical methods and assessment criteria for multi-ingredient RTE processed food samples requiring refrigeration

Bacterial analysis	Method identification number <sup>a</sup>	Satisfactory	Investigative	Unsatisfactory
Salmonella spp.	MFHPB-20	Absent in 25g	Not Applicable (N/A)	Present in 25g
S. aureus	MFHPB-21	$\leq 10^2  \text{CFU/g}$	$10^2 < x \le 10^4 \text{ CFU/g}$	> 10⁴ CFU/g
B. cereus	MFLP-42	≤ 10 <sup>3</sup> CFU/g	10 <sup>3</sup> < x ≤ 10 <sup>4</sup> CFU/g	> 10 <sup>4</sup> CFU/g
C. perfringens	MFHPB-23	$\leq 10^2 \text{ CFU/g}$	$10^2 < x \le 10^3 \text{ CFU/g}$	> 10 <sup>3</sup> CFU/g
L. monocytogenes	MFHPB-30 MFLP-28 MFLP-74	Al	N/A (Category 1 b)	Present in 25g (Category 1 <sup>b</sup> )
		Absent in 25g	Present and ≤ 10 <sup>2</sup> CFU/g (Category 2 <sup>b</sup> )	> 10 <sup>2</sup> CFU/g (Category 2 <sup>b</sup> )
Generic <i>E. coli</i>	MFHPB-19 MFHPB-27	≤ 10 <sup>2</sup> MPN/g or CFU/g	N/A	> 10 <sup>2</sup> MPN/g or CFU/g

At the time of writing this report, no assessment guidelines had been established in Canada for the presence of bacterial pathogens or indicator organisms in the RTE food types analysed in this survey. As Salmonella spp. is considered pathogenic to humans their presence was considered to be a violation of the Food and Drugs Act (FDA) Section 4(1)a<sup>12</sup> and therefore was assessed by the CFIA as unsatisfactory. The assessment guidelines for L. monocytogenes are based on Health Canada's Policy on Listeria monocytogenes in RTE foods 13 and are dependent upon the sample type analysed (Category 1, 2A or 2B).

S. aureus, C. perfringens and B. cereus are commonly found in the environment and are bacteria that can produce protein toxins when present in high levels in foods or in the intestines of infected humans, which can cause foodborne illness. An investigative assessment which may result in further follow-up actions is associated with elevated levels (table 1). As the results are based on the analysis of one unit (n=1), further sampling may be required to verify their levels in the lot. The presence of high levels of these organisms (table 1) is indicative of the potential to cause foodborne illnesses. Therefore, samples with high levels of S. aureus, C. perfringens or

<sup>&</sup>lt;sup>a</sup> The methods used were the published versions at the time of analysis
<sup>b</sup> The pH and water activity of the sample were used to determine the product Category

*B. cereus* (table 1) are assessed as unsatisfactory indicating that follow-up activities are warranted. The *B. cereus* method used in this survey is unable to discriminate *B. cereus* from other closely related organisms and therefore results are considered presumptive for *B. cereus*.

Unlike harmful bacterial pathogens (such as *Salmonella spp.*), generic *E. coli* is commonly found in the intestines of humans and most strains are harmless. It is considered to be an indicator organism and levels of generic *E. coli* found in foods are used to assess the overall sanitation conditions throughout the food chain from production to the point of sale. An unsatisfactory assessment is associated with levels of generic *E.coli* exceeding 10<sup>2</sup> MPN/g or CFU/g as it may indicate a breakdown in good manufacturing practices, and therefore possibly warranting the initiation of follow-up activities to determine the source of contamination and improve sanitation conditions along the food chain.

## What were the survey results?

Over the course of this four year study (April 1, 2013 to March 31, 2014 and April 1, 2015 to March 31, 2018), a total of 4851 samples were collected from retail locations in 11 cities across Canada. Throughout the entire four year survey period, all samples were tested for generic *E.coli*, *L. monocytogenes* and *Salmonella* spp.. Throughout the second, third and fourth year of the study, 3282 samples were tested for *B. cereus*, *C. perfringens*, and *S. aureus*.

*C. perfringens*, *Salmonella* spp. and generic *E.coli* (>  $10^2$  MPN/g or CFU/g) were not found in any samples. Presumptive *B. cereus* was detected at elevated levels ( $10^3 < x \le 10^4$  CFU/g) in 2/3282 (0.06%) of the samples. S. aureus was detected at elevated levels ( $10^2 < x \le 10^4$  CFU/g) in 1/3282 (0.03%) samples. *L. monocytogenes* was detected in 5/4851 (0.1%) samples, of which three were considered Category 1 and two were considered Category 2.

Table 2 - Assessment results of various multi-ingredient RTE processed food

samples requiring refrigeration by survey year

Survey year(s)	Bacterial analysis	Number of samples tested	Satisfactory	Investigative	Unsatisfactory
	L. monocytogenes			0	1
1	Salmonella spp.	1569	1568	N/A	0
	Generic <i>E.coli</i>			N/A	0
2-4	Salmonella spp.	3282		N/A 0 1 0 2 a 2 2 a 0 N/A 0	0
	S. aureus				0
	L. monocytogenes		3276		2
	B. cereus		3270		0
	Generic <i>E.coli</i>				0
	C. perfringens			0	0
Total		4851	4844	4	3

<sup>&</sup>lt;sup>a</sup> L.monocytogenes and B.cereus detected in one sample

Of the 4851 samples tested, 2489 (51%) were domestic and 1281 (26%) were imported (table 4). The country where the products were processed could not be determined (unknown origin) for 1081 (22%) samples (table 4).

Table 3 – Assessment results of various multi-ingredient RTE processed food samples requiring refrigeration by product origin

Product origin	Number of samples tested (% of total samples)	Satisfactory	Investigative B.cereus $(10^3 < x \le 10^4)$ CFU/g)	Investigative L. monocytogenes (present and $\leq 10^2$ CFU/g - Category 2)	Investigative S. aureus $(10^2 < x \le 10^4$ CFU/g)	Unsatisfactory L. monocytogenes (present in 25g - Category 1)
Domestic	2489 (51%)	2487	0	1	0	1
Import	1281 (26%)	1280	0	0	0	1
Australia	1	1	0	0	0	0
Costa Rica/Brazil/Ecuador	1	1	0	0	0	0
Israel	6	6	0	0	0	0
Japan	1	1	0	0	0	0
Jordan	2	2	0	0	0	0
Mexico	53	53	0	0	0	0
South Africa	1	1	0	0	0	0
United States	1213	1212	0	0	0	1
Unknown	3	3	0	0	0	0
Unknown	1081 (22%)	1077	2 <sup>a</sup>	1 <sup>a</sup>	1	1
Total	4851	4844	2	2	1	3

<sup>a</sup> L.monocytogenes and B.cereus detected in one sample

A variety of RTE product types were analysed (table 4).

Table 4 – Assessment results of various multi-ingredient RTE processed food

samples requiring refrigeration by product type

		<u> </u>	71			
Product type	Number of samples tested	Satisfactory	Investigative B.cereus $(10^3 < x \le 10^4 \text{ CFU/g})$	Investigative L. monocytogenes (present and $\leq 10^2$ CFU/g - Category 2)	Investigative S. aureus $(10^2 < x \le 10^4 \text{ CFU/g})$	Unsatisfactory L. monocytogenes (present in 25g - Category 1)
Dressing and dip	233	233	0	0	0	0
Dips	1264	1261	1 <sup>a</sup>	2 <sup>a</sup>	0	1
Salads (potato, pasta, etc.)	2270	2266	1	0	1	2
Salad dressing	609	609	0	0	0	0
Sauce	456	456	0	0	0	0
Sauce and salad dressing	19	19	0	0	0	0
Total	4851	4844	2	2	1	3

<sup>&</sup>lt;sup>a</sup> L. monocytogenes and B.cereus detected in one sample

Further details about each investigative and unsatisfactory sample are provided in table 5.

Table 5 – Detailed information about investigative and unsatisfactory multiingredient RTE processed food samples requiring refrigeration

g an entre : = p e e e a e e e e e e e e e e e e e e e						
	Investigative					
S. aureus Farfalle salad Unknown country of origin	L. monocytogenes B. cereus enumeration Hummus dip (category 2) Unknown country of origin	B. cereus Wild rice salad Unknown country of origin				
L. monocytogenes Hummus dip (category 2) Domestic	7					
Unsatisfactory						
L. monocytogenes Guacamole dip (category 1) Domestic	L. monocytogenes Chickpea salad (category 1) Unknown country of origin	L. monocytogenes Macaroni salad (category 1) Imported from the United States				

### What do the survey results mean?

In this survey, all (100%) of the multi-ingredient RTE processed food samples tested were free of *Salmonella* spp., *C. perfringens* and generic *E.coli* (>  $10^2$  MPN/g or CFU/g). Presumptive *B. cereus* was detected at elevated levels ( $10^3 < x \le 10^4$  CFU/g) in 2/3282 (0.06%) of the samples. *S. aureus* was detected at elevated levels ( $10^2 < x \le 10^4$  CFU/g) in 1/3282 (0.03%) samples and *L. monocytogenes* was detected in 5/4851 (0.1%) samples, of which three were considered Category 1 and two were considered Category 2.

Only a few studies similar to ours have been published as most studies on the microbiological quality of RTE foods focus on minimally processed RTE vegetable salads. One Danish study published in 2005 investigated the presence of *B. cereus* (and *Bacillus thuringiensis*) in retail RTE foods. The study looked at 48 901 samples of fresh fruits and vegetables, heat-treated products (such as sauces) and products with both fresh and heat-treated ingredients (such as sandwiches). The study found that 0.7% of sauces (n=4288), 1.2% of pasta salads (n=593), and 0.9% dressings (n=696) had counts of *B. cereus*-like organisms of  $10^3 < x \le 10^4$  CFU/g. The study also found that 0.4% of sauces (n=4288), 0.3% of pasta salads (n=593), and 0.1% of dressings (n=696) had counts of *B. cereus*-like organisms of >  $10^4$  CFU/g.

A study conducted in 2007<sup>15</sup> which investigated the microbiological quality of sauces (n=1208) from kebab take-away restaurants in the UK detected the following:

- E.coli (≥ 10<sup>2</sup> CFU/g) in 0.7% samples
- S. aureus (>100 to 10000 CFU/g) in 0.2% samples (3/1208)
- B. cereus and/or other pathogenic Bacillus species (≥10<sup>4</sup> to 10<sup>5</sup> CFU/g) in 3.8% samples (46/1208)
- B. cereus and/or other pathogenic Bacillus species (≥10<sup>5</sup> CFU/g) in 0.5% samples (6/1208)
- Salmonella Agbeni in 0.1% samples (1/1208)

No trends were observed in our study when comparing domestic vs. imported product (table 3) or product type (table 4). It is however interesting to note all (3/3) of the investigative dip samples were hummus (table 5). A total of 309 hummus samples were tested.

The CFIA conducted appropriate follow-up activities such as facility inspections and additional sampling. Recalls were issued in response to those Category 1 *L. monocytogenes* positive samples where the affected product was still on the market. The source of contamination was determined in one case and preventive and corrective actions were implemented by the manufacturing facilities. No reported illnesses were associated with any of the contaminated products.

Overall, our survey results suggest that almost all multi-ingredient RTE processed foods available for purchase at retail in Canada are safe for consumption. They can however be found to be contaminated with pathogens such as those found in this study: *B. cereus, S. aureus* and *L. monocytogenes*. Consequently, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

#### References

- 1. Public Health Agency of Canada, Foodbook Report. 2015.
- 2. Canadian Food Inspection Agency. *Food Recall Warnings*. Available from: <a href="http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/eng/1299076382077/1299076493846">http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/eng/1299076382077/1299076493846</a>.
- 3. Centers for Diease Control and Prevention, *Outbreaks of Salmonella Serotype Enteritidis Infection Associated with Consumption of Raw Shell Eggs United States*, 1994-1995. 1996. p. 737-742.
- 4. Schmid, D., et al., Salmonella Enteritidis phage type 21 outbreak in Austria, 2005. Eurosurveillance, 2006. 11(2): p. 67-9.
- 5. Juliao, P., et al., *National outbreak of type a foodborne botulism associated with a widely distributed commercially canned hot dog chili sauce.* Clinical Infectious Diseases, 2013. 56(3): p. 376-82.
- 6. Ercoli, L., et al., *Investigation of a Staphylococcal Food Poisoning Oubreak from a Chantilly Cream Dessert, in Umbria (Italy).* Foodborne Pathogens and Disease, 2017. 14(7): p. 407-413.
- 7. Health Canada, Compendium of Analytical Methods. 2011.
- 8. Hong Kong Centre For Food Safety, *Microbiological Guidelines for Food (for Ready-to-Eat food in General and Specific Food Items)*. 2014.
- 9. United Kingdom Health Protection Agency, Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. 2009.
- 10. New South Wales Food Authority, *Microbiological Quality Guide for Ready-to-Eat Food*. 2009.
- 11. International Life Science Institute Europe, *The Enterobacteriaceae and Their Significance to the Food Industry*. 2011.
- 12. Department of Justice Canada, Food and Drugs Act. 2014.
- 13. Health Canada, Policy on Listeria monocytogenes in Ready-to-Eat Foods. 2011.
- 14. Rosenquist, H., et al., Occurrence and significance of Bacillus cereus and Bacillus thuringinensis in ready-to-eat food. FEMS Microbiology Letters, 2005. 250: p. 129-136.
- 15. Meldrum, R.J., et al., Assessment of the microbiological safety of salad vegetables and sauces from take-away restaurants in the United Kingdom. Food Microbiology, 2009. 26: p. 573-577.