

# Bacterial Pathogens in Raw Milk Cheese - November 30, 2014 to March 31, 2018

Food microbiology - Targeted surveys - Final report





### **Summary**

Raw milk cheeses are consumed by Canadians in all age groups. Unfortunately, they have been associated with numerous outbreaks of foodborne illnesses in Canada and worldwide. The main food safety concern with raw milk cheeses is the use of raw milk that has been contaminated with pathogens. Contamination can also occur during handling, packaging and distribution. Currently, all raw milk cheeses sold in Canada must be aged for at least 60 days as this process is believed to reduce the likelihood of pathogen survival. Since raw milk cheeses are ready-to-eat (RTE) foods, the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and in response to a 2013 *Escherichia coli* O157 (*E.coli* O157) outbreak associated with Canadian raw milk cheese, raw milk cheeses were selected for targeted surveys. The purpose of the surveys was to generate baseline information on the occurrence of pathogenic bacteria in raw milk cheeses. Over the course of this study (November 30, 2014 to March 31, 2018), a total of 2009 samples were collected from retail locations in 11 cities across Canada. All 2009 samples were tested for *E.coli* O157. Of the 2009 samples, 1723 samples were tested for generic *Escherichia coli* (*E.coli*) and the pathogens *Salmonella* species (spp.), *Staphylococcus aureus* (*S. aureus*) and *Listeria monocytogenes* (*L. monocytogenes*). 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.

Salmonella spp, E.coli O157 and generic E.coli (>500 Most Probable Number (MPN)/ gram (g) or Colony Forming Units (CFU)/g) were not found in any samples. S. aureus was detected at elevated levels ( $10^3 < x \le 10^4$  CFU/g) in 4/1723 (0.2%) samples and at high levels (> $10^4$  CFU/g) in 2/1723 (0.1%) samples. L. monocytogenes was detected in 2/1723 (0.1%) samples.

In raw milk cheeses the presence of elevated levels  $(10^3 < x \le 10^4 \text{ CFU/g})$  of *S. aureus* indicate that the food may have been produced under unsanitary conditions. When *S. aureus* is present in high levels  $(>10^4 \text{ CFU/g})$ , the samples are considered to 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 the detection of *L. monocytogenes* and *S. aureus* respectively. The source of contamination was determined in some cases and preventive and corrective actions were implemented by the facilities.

Overall, our survey results suggest that almost all raw milk cheeses are safe for consumption. However, those susceptible to foodborne illness such as children, elderly, pregnant women and

people with weakened immune systems are advised to avoid their consumption as they can be found to be contaminated with pathogens such as those found in this study: *S. aureus* and *L. monocytogenes*. Additionally, 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

Raw milk cheeses are consumed by Canadians in all age groups<sup>1</sup>. Unfortunately, they have been associated with numerous recalls and outbreaks of foodborne illnesses in Canada<sup>2,3,4</sup> and worldwide<sup>5,6,7</sup>. The main food safety concern with raw milk cheeses is contamination by pathogens present in the raw milk during production. Contamination can also occur during handling, packaging and distribution. Currently, all raw milk cheeses sold in Canada must be aged for at least 60 days as this process is believed to reduce the likelihood of pathogen survival. Since raw milk cheeses are RTE foods, the presence of bacterial pathogens creates a potential risk for foodborne illnesses.

Considering the factors mentioned above and in response to a Canadian raw milk cheese associated outbreak in 2013, raw milk cheeses were selected for targeted surveys from November 30, 2014 to March 31, 2018. This survey was implemented to gain further information on the occurrence of generic *E.coli* and the pathogens E.coli O157, *Salmonella* spp., *S. aureus* and *L. monocytogenes* 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 4 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 November 30, 2014 and March 31, 2018.

A variety of domestic, imported, conventional and organic raw milk cheeses were sampled. In the first four months of the survey (November 30, 2014 to March 31, 2015), the ratio of domestic to imported samples was 1:2 as the majority of raw milk cheeses sold in Canada are imported. In the remaining three years of the survey (April 1, 2015 to March 31, 2018), the ratio of domestic to imported samples was 1:5. 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>8</sup> (table 1). The assessment criteria used in this survey (table 1) are based on the principles of Health Canada's *Health Products and Food Branch Standards and Guidelines for Microbiological Safety of Foods*<sup>9</sup>.

Table 1 - Analytical methods and assessment criteria for raw milk cheese

samples

Bacterial analysis	Method identification number <sup>a</sup>	Satisfactory	Investigative	Unsatisfactory	
Salmonella spp.	MFHPB-20 MFLP-40 MFLP-29	Absent in 25g	Not Applicable (N/A)	Present in 25g	
S. aureus	MFHPB-21	≤ 10 <sup>3</sup> CFU/g	$10^3 < x \le 10^4 \text{ CFU/g}$	> 10 <sup>4</sup> CFU/g	
E. coli O157	MFLP-30 MFLP-80 MFHPB-10	Absent in 25g	N/A	Present in 25g	
L.	MFHPB-30 MFLP-74	About in 25g	N/A (category 1 <sup>b</sup> )	Present in 25g (category 1 <sup>b</sup> )	
monocytogenes	MFLP-77	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 E. coli	MFHPB-19 MFHPB-34	≤ 5x10² MPN/g or CFU/g	$5x10^2 < x \le 2x10^3$ MPN/g or CFU/g	> 2x10 <sup>3</sup> MPN/g or CFU/g	

<sup>&</sup>lt;sup>a</sup> The methods used were the published versions at the time of analysis

At the time of writing of this report, no assessment guidelines had been established in Canada for the presence of *Salmonella* spp., *E.coli* O157, or *L. monocytogenes* in raw milk cheeses. As *Salmonella* spp. and *E.coli* O157 are considered pathogenic to humans their presence was considered to be a violation of the *Food and Drugs Act* (FDA) Section 4(1)a<sup>10</sup> and was therefore 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 and is dependent upon the sample type analysed (category 1, 2A or 2B)<sup>11</sup>.

*S. aureus* is commonly found in the environment and 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 ( $10^3 < x \le 10^4$  CFU/g). 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 *S. aureus* (> $10^4$  CFU/g) is indicative of the potential to cause foodborne illnesses. Therefore, samples with high levels were assessed as unsatisfactory indicating that follow-up activities are warranted.

<sup>&</sup>lt;sup>b</sup> The pH and water activity of the sample were used to determine the product category

Unlike harmful bacterial pathogens (such as *Salmonella* spp., *E. coli* O157), generic *E. coli* is commonly found in the intestines of humans and most strains are harmless. It is considered to be an indicator organism as 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. Its presence at some levels is tolerated on agricultural products. An investigative assessment which may result in further follow-up actions is associated with elevated levels of generic *E. coli*  $(5x10^2 < x \le 2x10^3 \text{ MPN/g} \text{ or CFU/g})$ . As the results are based on the analysis of one unit (n=1), further sampling may be required to verify the levels of generic *E. coli* of the lot. An unsatisfactory assessment is associated with high levels of generic *E.coli* (>  $2x10^3 \text{ MPN/g}$  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 the contamination and improve sanitation conditions along the food chain.

### What were the survey results

Over the course of this study (November 30, 2014 to March 31, 2018), a total of 2009 samples were collected and tested for bacterial pathogens of concern as well as generic *E. coli*. All 2009 samples were tested for *E.coli* O157 throughout the entire three year four month survey period. 1723 samples were tested for *Salmonella* spp., *S. aureus*, *L. monocytogenes* and generic *E.coli* during the last three years (April 1, 2015 to March 31, 2018) of the survey period.

Salmonella spp., E.coli O157 and generic E.coli (>500 MPN/g or CFU/g) were not found in any samples. S. aureus was detected at elevated levels (10³<x≤10⁴ CFU/g) in 4/1723 (0.2%) samples and at high levels (>10⁴ CFU/g) in 2/1723 (0.1%) samples. L. monocytogenes was detected in 2/1723 (0.1%) samples which were Category 1 products¹¹. Sample assessment results can be found in table 2.

Table 2 - Assessment results of raw milk cheese samples

Survey period	Bacterial analysis	Number of samples tested	Satisfactory	Investigative	Unsatisfactory
November 30, 2014 to March 31, 2015	E. coli O157	286	286	N/A	0
April 1, 2015 to March 31, 2018	Salmonella spp.		1715	N/A	0
	S. aureus			4 <sup>c</sup>	2 <sup>c</sup>
	L. monocytogenes	1723		0	2
	E. coli O157			N/A	0
	Generic <i>E.coli</i>			0	0
Total		2009	2001	4	4

<sup>&</sup>lt;sup>c</sup> 4/4 Investigative and 1/2 Unsatisfactory were St. Jorge cheese, of which 4 (3 Investigative and 1 unsatisfactory) samples were manufactured by the same company and 3 from the same lot.

Of the 2009 samples tested, 1869 (93%) were conventional and 140 (7%) were organic (table 3).

Table 3 – Assessment results of raw milk cheese samples by production practice

Production practice	Number of samples tested (% of total samples)	Satisfactory	Investigative S. aureus (10 <sup>3</sup> < x ≤ 10 <sup>4</sup> CFU/g)	Unsatisfactory L. monocytogenes (Present in 25g - Category 1)	Unsatisfactory S. aureus (> 10 <sup>4</sup> CFU/g)
Conventional	1869 (93%)	1861	4 <sup>d</sup>	2	2 <sup>d</sup>
Organic	140 (7%)	140	0	0	0
Total	2009	2001	4	2	2

<sup>&</sup>lt;sup>d</sup> 3/4 Investigative and 2/2 Unsatisfactory samples were St. Jorge cheese. 4/6 (3 Investigative and 1 unsatisfactory) samples were manufactured by the same company, of which 3 were from the same lot.

Of the 2009 samples tested, 390 (19%) were domestic and 1619 (81%) were imported (table 4).

Table 4 – Assessment results of raw milk cheese samples by country of origin

Product origin	Number of samples tested (% of total samples)	Satisfactory	Investigative S. aureus (10³ < x ≤ 10⁴ CFU/g)	Unsatisfactory L. monocytogenes (Present in 25g - Category 1)	Unsatisfactory S. aureus (> 10 <sup>4</sup> CFU/g)
Domestic	390 (19%)	389	1	0	0
Import	1619 (81%)	1612	3	2	2
Belgium	1	1	0	0	0
France	245	243	0	2	0
Italy	629	629	0	0	0
Netherlands	1	1	0	0	0
Portugal	16	11	3 <sup>e</sup>	0	2 e
Spain	1	1	0	0	0
Switzerland	699	699	0	0	0
United States	27	27	0	0	0
Total	2009	2001	4	2	2

<sup>&</sup>lt;sup>e</sup> 5/5 samples were St. Jorge cheese. 4/5 (3 Investigative and 1 unsatisfactory) samples were manufactured by the same company, of which 3 were from the same lot.

A variety of raw milk cheese product types were analysed (table 5). The cheeses are classified according to their moisture on a fat free basis (MFFB)<sup>12</sup>.

Table 5 – Assessment results of raw milk cheese samples by MFFB

MFFB	Number of samples analysed (% of Total Samples)	Satisfactory	Investigative S. aureus (10³ < x ≤ 10⁴ CFU/g)	Unsatisfactory L. monocytogenes (Present in 25g - Category 1)	Unsatisfactor y S. aureus (> 10 <sup>4</sup> CFU/g)	
Hard (<50%)	626 (31%)	626	0	0	0	
Firm (50- 62%)	1215 (61%)	1207	4 <sup>f</sup>	2	2 <sup>f</sup>	
Semi-soft (62-67%)	158 (8%)	158	0	0	0	
Soft (67-80%)	10 (<0.5%)	10	0	0	0	
Total	2009	2001	4	2	2	

f 3/4 Investigative and 2/2 Unsatisfactory samples were St. Jorge cheese. 4/6 (3 Investigative and 1 unsatisfactory) samples were manufactured by the same company, of which 3 were from the same lot.

Further details about each investigative and unsatisfactory sample are provided in table 6.

Table 6 – Detailed information about investigative and unsatisfactory raw milk cheese samples

Investigative S. aureus (10³ < x ≤ 10⁴ CFU/g)	Investigative S. aureus (10 <sup>3</sup> < x ≤ 10 <sup>4</sup> CFU/g)	Unsatisfactory S. aureus (> 10 <sup>4</sup> CFU/g)	Unsatisfactory L. monocytogenes (Present in 25g - Category 1)
St. Jorge (n=3) Firm Conventional Imported from Portugal	Cheddar (n=1) Firm Conventional Domestic	St. Jorge (n=2) Firm Conventional Imported from Portugal	Comte (n=2) Firm Conventional Imported from France

### What do the survey results mean

In this survey, all (100%) of the raw milk cheese samples tested were free of *Salmonella* spp., *E. coli* O157 and generic *E.coli* (>500 MPN/g or CFU/g). *S. aureus* was detected at elevated levels (10³<x≤10⁴ CFU/g) in 4/1723 (0.2%) samples and at high levels (>10⁴ CFU/g) in 2/1723 (0.1%) samples. *L. monocytogenes* was detected in 2/1723 (0.1%) samples, which were Category 1 products¹¹.

Studies similar in scale to ours were conducted in the US<sup>13</sup> and UK<sup>14, 15</sup>. Similar to our study, *E. coli* O157 was not detected in any of the studies. *Salmonella* spp. was not detected in our or the UK<sup>14</sup> study, however it was detected in 0.19% of the samples in the US<sup>13</sup> study. *L. monocytogenes* and *S. aureus* were found at lower rates in our study as compared to the other studies. Differences in the bacterial detection rates between studies may be attributable to various factors such as differences in study design, product types tested and/or testing methodologies used. See table 7 for a summary.

Smaller scale studies were also conducted in the US and Italy. Similar to our study, *Salmonella spp.* and *E.coli* O157 were not detected in any of the studies<sup>16, 17</sup>. *L. monocytogenes* was detected in 0.12% of the samples in the current study, however it was not detected in the other studies<sup>16, 17</sup>. *S. aureus* was detected in the US study<sup>17</sup> at a higher rate as compared to our study. Differences in the bacterial detection rates between studies may be attributable to various factors such as differences in study design, product types tested and/or testing methodologies used. See table 7 for a summary.

Table 7 – Comparison of prevalence of bacterial pathogens and generic *E.coli* in various

raw milk cheese surveys

raw milk cheese	raw miik cheese surveys								
Bacterial analysis	Canada <sup>9</sup> - retail (2014 to 2018)	USh - manufacturers, distributors, ports of entry, retail (2014 to 2015)	UK <sup>i</sup> - retail (2004 to 2005)	UK <sup>i</sup> – retail (1997 to 1998)	Italy <sup>k</sup> - retail (2009)	US <sup>I</sup> - retail (2012)			
Salmonella	0/1723	3/1606	0/1819	n/a	n/a	0/41			
L. monocytogene s	2/1723	10/1606	16/1819 ≤100 CFU/g 1/1819 >100 CFU/g	n/a	0/50	0/41			
E.coli O157	0/2009	0/1606	n/a	0/739	n/a	0/41			
Non-O157 VTEC	n/a	12/1606	n/a	n/a	n/a	n/a			
S. aureus	4/1723 10 <sup>3</sup> <x≤10<sup>4 CFU/g 2/1723 &gt;10<sup>4</sup> CFU/g</x≤10<sup>	n/a	13/1819 10 <sup>3</sup> <x≤10<sup>4 CFU/g 13/1819 &gt;10<sup>4</sup> CFU/g</x≤10<sup>	n/a	n/a	1/41 10³ <x≤ 10⁴<br="">CFU/g 2/41 &lt;10² CFU/g</x≤>			
Generic <i>E.coli</i>	0/1723 >500 CFU/g	87/1606 10 <x≤10² cfu="" g<br="">in three or more</x≤10²>	26/1819 10 <sup>4</sup> <x≤10<sup>5 CFU/g</x≤10<sup>	725/735 <10 <sup>4</sup> CFU/g	4/50 10 <sup>2</sup> <x≤1 0<sup>3</sup> CFU/g</x≤1 	2/41 <100			
	2500 Ci 0/g	of five subsamples	25/1819 >10 <sup>5</sup> CFU/g	10/735 >10 <sup>4</sup> CFU/g	22/50 >10 <sup>3</sup> CFU/g	CFU/g			

<sup>&</sup>lt;sup>9</sup>Current Study

In a recent study conducted in the US<sup>13</sup>, 1606 samples of raw milk cheese aged 60 days were collected from manufacturers, distribution centres, ports of entry and retail stores. These samples were tested for the presence of *Salmonella*, *L. monocytogenes*, *E. coli* O157, non-O157 Verotoxigenic *E.coli* (VTEC) and generic *E.coli*. The study found *Salmonella* in 0.19% (3/1606) and *L. monocytogenes* in 0.62% (10/1606) of the samples. The study did not detect *E. coli* O157 in any of the samples, however it did detect non-O157 VTEC in 0.68% (12/1606) of the samples, of which 1 (0.06%) was determined to be pathogenic. Finally, the study found

<sup>&</sup>lt;sup>h</sup>United States Food and Drug Administration, *FY 2014-2016 Microbiological Sampling Assignment Summary Report: Raw Milk Cheese Aged 60 Days.* 2016.

Little, C.L., et al., *Microbiological quality of retail cheeses made from raw thermised or pasteurized milk in the UK.* Food Microbiology. 2008. 25(2): p. 304-312.

Coai, J.E., et al., A survey of the prevalence of E.coli O157 in raw meats, raw cow's milk and raw-milk cheeses in south-east Scotland. International Journal of Food Microbiology. 2001. 66(1-2): p. 63-69. 

kGiammanco, G.M., et al., Microbiological quality of Pecorino Siciliano "Primosale" cheese on retail sale in the street markets of Palermo, Italy. New Microbiologica. 2011. 34: p. 179-185.

J.C. Brooks, B.M., et al., Survey of raw milk cheeses for microbiological quality and prevlance of foodborne pathogens. Food Microbiology. 2012. 31: p. 154-158.

87/1606 (5.4%) to be violative (10 <  $x \le 10^2$  CFU/g in three or more of five subsamples tested) for the presence of generic *E.coli*.

From 2004 to 2005, another study was conducted in the UK<sup>14</sup> which investigated the microbiological quality of retail raw milk cheeses (n=1819). The study detected elevated ( $10^3 < x \le 10^4$  CFU/g) and high levels of *S. aureus* ( $\ge 10~000$  CFU/g) in 0.7% (13/1819) and 0.7% (13/1819) of the samples analysed respectively. The study also detected elevated ( $10^4 < x \le 10^5$  CFU/g) and high levels of generic *E.coli* ( $\ge 10^5$  CFU/g) in 1.4% (26/1819) and 1.3% (25/1819) respectively of the samples analysed. *L. monocytogenes* was detected at  $\le 100$  CFU/g in 0.9% (16/1819) of samples and at  $\ge 100$  CFU/g in 0.05% (1/1819) of the samples analysed. *Salmonella* was not detected in any of the samples.

From 1997 to 1998 a study was conducted in the UK<sup>15</sup>, which investigated the presence of *E.coli* O157 and generic *E.coli* in raw-milk cheeses. The raw-milk cheese samples (n=739) were collected from retail outlets. *E.coli* O157 was not detected in any of the raw milk cheese samples analysed. Of the 739 samples analysed, 735 samples produced an available generic *E.coli* count. 725/735 samples had a count of <10 000 CFU/g while 10/735 had counts greater than 10 000 CFU/g (ranging from 19 500 CFU/g to 230 454 CFU/g).

The US study (published in 2012)<sup>17</sup> investigated the microbiological quality of 41 raw milk cheeses collected from retail stores (n=29), farmers' markets (n=6) and on-line sources (n=6). Of the 41 samples analysed, generic *E.coli* (<100 CFU/g) was detected in 2 samples (4.9%), *S. aureus* at levels <100 CFU/g were detected in 2 samples (4.9%), *S. aureus* at levels >1 000 CFU/g was found in 1 sample (2.4%). *Listeria* spp., *Salmonella* spp., and *E.coli* O157:H7 were not detected in any of the samples.

The Italian study was conducted in  $2009^{16}$  and investigated the microbiological quality of street vended Percorino Siciliano raw milk cheese (n=50) samples. Generic *E.coli* was found at levels  $10^2 < x < 10^3$  CFU/g in 8% (4/50) samples and at levels  $\ge 10^3$  CFU/g in 44% (22/50) samples. *L. monocytogenes* was not detected in any samples.

No trends were observed in our study with respect to production practice (table 3), when comparing domestically vs. imported product (table 4) or MFFB (table 5). It should however be noted that a high percentage of the samples imported from Portugal were found to be investigative (18%, 3/17) and unsatisfactory (12%, 2/17). This was partly due to the same cheese type (St. Jorge) from Portugal being sampled in multiple retail stores and on multiple dates throughout our study. Of the investigative (n=3) and unsatisfactory (n=2) St. Jorge samples, 4 were manufactured by the same company and of those, 3 were from the same lot.

The CFIA conducted appropriate follow-up activities such as facility inspections and additional sampling. Recalls were issued in response to the detection of *L. monocytogenes* and *S. aureus* 

respectively. The source of contamination was determined in some cases and preventive and corrective actions were implemented by the facilities.

Overall, our survey results suggest that almost all raw milk cheeses are safe for consumption. However, those susceptible to foodborne illness such as children, elderly, pregnant women and people with weakened immune system are advised to avoid their consumption as they can be found to be contaminated with pathogens such as those found in this study: *S. aureus* and *L. monocytogenes*. Additionally, as with all foods, safe handling practices are recommended for producers, retailers and consumers.

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