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Consuming fresh produce is healthy, but if berries and leafy greens are contaminated with the parasite, *Cyclospora*, this can cause gastroenteritis. Read how identifying the source of a cylcosporiasis outbreak can be challenging. (https://www.shutterstock.com/image-photo/assortment-fresh-fruits-vegetables-berries-bunch-351003551)

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OUTBREAKS

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OUTBREAK REPORT

Use of a case–control study and control bank to investigate an outbreak of locally acquired cyclosporiasis in Canada, 2016

V Morton[1]*, K Meghnath[2], M Gheorghe[2], A Fitzgerald-Husek[3], J Hobbs[4], L Honish[5], S David[6]

Abstract

Background: *Cyclospora* is an intestinal parasite that is not endemic in Canada. However, national outbreaks of locally acquired cases have been reported since 2013. These outbreaks were suspected to be associated with consumption of produce imported from countries where *Cyclospora* is endemic. Identification of the source can be challenging because of reporting delays and limited traceability of produce.

Objective: To report on a national outbreak of locally acquired cyclosporiasis, highlight the challenges of investigating these outbreaks and document the first time use of a control bank to recruit controls for a national outbreak case–control study in Canada.

Methods: Cases of cyclosporiasis were identified through provincial laboratory testing and reported through provinces to the national level. Cases were interviewed about food exposures using a questionnaire and food exposures reported by cases were compared to Foodbook reference values. To narrow down the food items of interest, a matched case–control study was conducted. Controls for the study were recruited primarily from a control bank, that is, a list of individuals who had previously agreed to participate in public health–related surveys.

Results: In total, 87 cases of locally acquired cyclosporiasis with onset or report dates between May 19, 2016 and August 10, 2016 were reported by four provinces. Comparing case exposures to Foodbook reference values identified several food items of interest, including blackberries, other berries, herbs and leafy greens. The case–control study identified only blackberries and mesclun greens as significantly more frequently consumed by cases than controls. Due to lack of product details for blackberries and mesclun greens, the source of the outbreak was not conclusively identified.

Conclusion: Blackberries were the primary food item of interest, but could not be identified as the conclusive source due to lack of traceability. The control bank was found to be a useful tool for control recruitment.

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Keywords: *Cyclospora*, outbreak, case–control study, cyclosporiasis, Canada

Introduction

Cyclospora cayetanensis is an enteric parasite that causes gastroenteritis (1). *Cyclospora* is not endemic in Canada; cases are often associated with international travel. Locally acquired cases of cyclosporiasis are likely associated with consumption of produce imported from countries where *Cyclospora* is endemic (2). For instance, in the 1990s there were several outbreaks associated with raspberries imported from Guatemala; import restrictions put an end to these outbreaks (3). Since 2013, national outbreaks of locally acquired cyclosporiasis have occur in Canada each spring/summer. However, identification of the

source has been difficult because of lack of molecular typing methods, considerable case reporting delays and limited traceability of produce.

An important step in foodborne outbreak investigation is hypothesis generation, and one hypothesis generation technique is to compare food exposures reported by cases to exposures reported by the general population (4). In Canada, investigators can use Foodbook reference values for comparisons. Foodbook is a population-based telephone survey that was conducted in all Canadian provinces and territories over a one-year period (2014–2015) with a primary focus of describing what foods Canadians eat over a seven-day period. Case–control studies are also commonly used for hypothesis generation during outbreak investigations. However, they can be lengthy and costly to conduct. One of the challenges associated with these studies is having an effective method to recruit controls. A control bank was created as part of the Foodbook study in order to address this challenge. Participants were asked if they were willing to be contacted for future outbreak investigation or research studies. The use of a control bank to recruit study participants was successfully used in Australia for outbreak investigations and other public health research projects in Australia (5,6).

The objective of this report is to describe the investigation into a national outbreak of locally acquired cyclosporiasis in 2016 and highlight some of the challenges associated with cyclosporiasis outbreak investigation in Canada.

Outbreak detection

On June 27, 2016, public health officials in British Columbia notified the Public Health Agency of Canada (PHAC) of their first reported case of locally acquired cyclosporiasis that year. By July 9, 2016, Ontario and Alberta had reported eight locally acquired cases. PHAC, in collaboration with local, provincial and federal partners, promptly launched a national outbreak investigation.

Methods

Epidemiological investigation

An outbreak-associated case of cyclosporiasis was defined as laboratory confirmation of infection with *C. cayetanensis* in a resident of or visitor to Canada, with symptom onset on or after April 1, 2016, and no history of travel outside of Canada or the United States during the 14 days preceding symptom onset. Cases were identified per provincial diagnosing standards and reported to PHAC by provincial public health officials. Data on food exposures in the 14 days preceding illness onset were obtained using a cyclosporiasis-specific questionnaire. Certain cases were re-interviewed by centralized interviewers at the national or local level if the initial interviews did not obtain the required details on exposures or product. Data were collected on exposure to fresh berries, herbs, leafy greens, peas and other vegetables; only exposure to fresh produce was considered. Exposure frequencies were compared to food reference values from the Foodbook study for the months of May, June, July and August for the affected provinces using binomial probabilities and significance thresholds of p=0.05 (7,8).

Case–control study

A case–control study was conducted to identify food items associated with illness. Cases were eligible for inclusion in the study if they met the outbreak case definition and had both a reasonably complete questionnaire and a known date of illness onset. Cases also had to have an onset date after May 31, 2016, and the illness had to have been reported on or before September 26, 2016. Controls were matched to each case based on age group (10–19, 20–69 and ≥70 years) and location using the first digit of the postal code (9). Controls were asked about food exposures during the same 14-day period as cases. Three controls were selected for each case in order to maximize the statistical power of the results based on the estimated sample size. Controls were recruited using a control bank, a repository of contact information for people who were interviewed for the Foodbook study and who had consented to be contacted for future research or investigative purposes. Additional recruitment, once the control bank was exhausted, was conducted via random digit dialling.

Case–control data were analyzed using McNemar's odds ratios (ORs) for matched pairs. Factors identified in univariable analysis with ORs greater than one and a p-value of less than 0.2 were included in a multivariable regression model. Factors were removed using backward stepwise selection; variables remained in the model if they changed the significant coefficients by more than 20% (10,11).

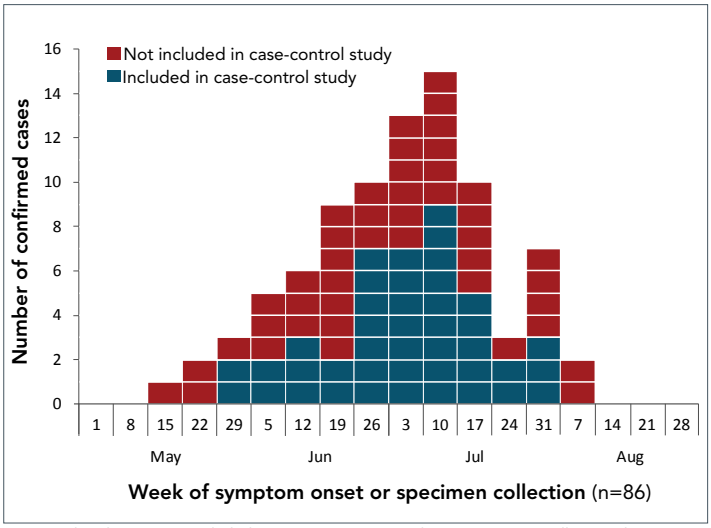
Results

Epidemiological investigation

In total, 87 cases of locally acquired cyclosporiasis were identified in four provinces (Ontario, n=75; Quebec, n=8; Alberta, n=2; British Columbia, n=2). Onset or specimen collection dates ranged from May 19 to August 10, 2016 (**Figure 1**). One case was excluded from the food history because of the uncertainty around the onset date (reported as "mid-July") and the long delay between the exposure period and interview (isolation date was September 23, 2016).

The median age of cases was 51 years (range = 15–89 years) and 52% were female. One case was hospitalized. There were no deaths.

Figure 1: Number of confirmed outbreak cases of locally acquired cyclosporiasis by symptom onset or specimen collection date and status of inclusion/exclusion in the case–control study, 2016, Canada[a]



[a] One outbreak case was excluded as no symptom onset date or specimen collection date was available; only an isolation date was available

Exposure data were available for 86 cases. The most frequently reported food items were romaine lettuce (51/70, 73%), strawberries (51/74, 69%) and other berries (51/74, 69%) (**Table 1**). Compared with Foodbook reference values, cases with cyclosporiasis reported the following seven food exposures significantly ($p \geq 0.05$) more frequently than did the general population: raspberries, blackberries, cilantro, parsley, romaine lettuce, spinach and mesclun greens.

Case–control study

Forty-two cases met the inclusion criteria for the case–control study. They were matched with 126 controls (117 from the control bank and nine enrolled via random digit dialling). The response rates for the control bank and random digit dialling were 60% and 24%, respectively.

Univariate matched analysis indicated that cases were significantly more likely than controls to have been exposed to blackberries (OR = 2.50; 95% confidence intervals [CI] = 1.16–4.91) and mesclun greens (OR = 2.50; 95% CI = 1.01–5.48) (Table 1). Multivariate analysis using backward stepwise selection resulted in only exposure to blackberries being significant, with exposure to mesclun greens and arugula included in the model as statistical confounders.

Table 1: Results of binomial probability comparison between Foodbook reference values and odds ratio results from case–control study

Food item	Foodbook reference (seven-day recall)[a]			Case–control study (14-day recall)[b]			
	Cases exposed (%)	Controls[c] exposed (%)	Binomial probability (*p*-value)	Cases exposed (%)	Controls exposed (%)	Odds ratio	*p*-value
Strawberries	69	64	0.07	68	79	0.57	0.16
Raspberries	51	30	<0.001	53	50	1.09	0.81
Blackberries	43	11	<0.001	49	28	2.50	0.02
Other berries	69	No data[d]	NA	73	59	1.86	0.12
Basil	23	22	0.11	24	35	0.60	0.23
Cilantro	42	19	<0.001	38	39	0.96	0.93
Parsley	47	33	0.01	53	39	1.78	0.16
Other fresh herbs	27	No data[d]	NA	32	24	1.48	0.37
Iceberg lettuce	39	44	0.06	44	64	0.45	0.03
Romaine lettuce	73	53	<0.001	76	84	0.60	0.27
Spinach	48	33	0.003	50	67	0.49	0.06
Mesclun greens	30	18	0.003	30	15	2.50	0.03
Arugula	42	No data[d]	NA	46	31	1.92	0.13
Other lettuce or leafy greens	43	No data[d]	NA	46	42	1.18	0.66
Peas	15	29	0.002	20	37	0.42	0.05
Snow peas	10	No data[d]	NA	11	26	0.35	0.17
Snap peas	17	No data[d]	NA	21	20	1.04	0.94

Abbreviations: NA, not applicable; <, inferior to
[a] Foodbook reference was based on all the cases (n=86)
[b] The case–control study was based on eligible cases (n=42) and controls (n=126)
[c] Foodbook controls were individuals aged over 10 years living in Alberta, British Columbia, Ontario or Quebec whose information was gathered via a population-based telephone survey conducted in all Canadian provinces and territories over a one-year period (2014–2015); this included those who were interviewed between May and September 2016 (unpublished study)
[d] Because these food items were not included in the Foodbook survey, the binomial probability was not calculated

Food safety investigation

Based on the results of the case–control study, attempts were made to trace food items that were significantly more likely to have been consumed by cases. Brand information, purchase dates, loyalty card data and store records were used. Import data were requested for blackberries and mesclun greens for May through August 2016; import data for mesclun greens were not available.

A common country of origin or supplier of blackberries was not identified based on the limited information available. Mexico was the largest supplier of blackberries in May, accounting for 64% of Canadian imports. This market share decreased to 20% in June and 3%–4% in July and August; during July and August the majority of blackberries imported into Canada were imported from the United States. Guatemala accounted for 0.2% of blackberry imports from May to August.

Public health response

The public was notified of the outbreak of locally acquired cyclosporiasis through a Public Health Notice posted on the PHAC website. As no specific product was identified, general prevention recommendations were provided. These recommendations encourage cooking produce imported from countries where *Cyclospora* is common and consuming fresh produce grown in countries where *Cyclospora* is not common. People travelling to a country where *Cyclospora* is found were advised to avoid food that has been washed in local drinking water, to drink water from a safe source and to eat cooked food or fruit that can be peeled.

Discussion

This outbreak investigation demonstrates some of the challenges associated with identifying the source of cyclosporiasis outbreaks. Despite a thorough investigation, the source of this outbreak was not identified. However, both the Foodbook exposure information and the case–control study were useful in generating hypotheses. To our knowledge, this outbreak was the first time a case–control study was conducted as part of an investigation into a national cyclosporiasis outbreak in Canada, and the control bank proved to be an effective tool for recruiting controls.

Although blackberries were not conclusively identified as the source of the outbreak, they were identified as a food item of interest via both the Foodbook reference and the case–control study. In addition, the case–control study helped to strengthen the evidence for blackberries as a suspect source. The reason these findings alone could not prove that blackberries were the source of the outbreak was the lack of specificity on the brand or producer of blackberries consumed by cases. These findings were consistent with previous locally acquired cyclosporiasis outbreaks in Canada in 2014 and 2015 where blackberries were identified as one of multiple suspected sources (*Public Health Agency of Canada. Multi-provincial outbreak investigation of Cyclosporiasis in British Columbia, Quebec and Ontario: Final Epidemiological Summary, unpublished report*). This finding is also supported by examination of blackberry import data, which revealed that Mexico was a large supplier of blackberries to Canada in May and early June. When taking into account the incubation period of cyclosporiasis (up to 14 days) and the shelf life of blackberries (up to 21 days), illnesses associated with these blackberries could have occurred more than a month after importation, lining up with the peak of the outbreak in July (3,12). *Cyclospora* is endemic in Mexico, and cyclosporiasis cases have been reported among Canadians travelling to Mexico (13). It is probable that one or more additional food items also contributed to this outbreak as only 43% of cases reported blackberry consumption and the percentage of cases who reported consuming blackberries decreased among those who reported later onset dates.

Both the Foodbook comparison and the case–control analysis identified mesclun greens as another item of interest. However, interpretation of this finding was limited by the lack of standardization in the types of greens identified as "mesclun greens" and a lack of packaging or brand details; this precluded positive identification of a specific product.

Strengths and limitations

This outbreak demonstrated the usefulness of a control bank for recruitment of controls for a case–control study. Having a list of persons willing to participate in surveys facilitated rapid and cost-effective initiation of a case–control study. In addition, the response rate for the control bank was much higher than random digit dialling; the decrease in household landline use and the increased availability of call display has made it more difficult to recruit individuals using random digit dialling. However, the use of a control bank introduces the possibility of selection bias as individuals who agree to participate in the control bank may differ from the general population.

One other important limitation to note is that the time periods for the food history for the case–control study data and the Foodbook data differed: Foodbook participants were asked about a seven-day food history, whereas cases in this investigation were asked about exposures over 14 days based on the *Cyclospora* incubation period. This difference in recall period could have resulted in more food items being reported at higher frequencies by the cases than the reference population from Foodbook.

Next steps

Case–control studies are a helpful tool in investigation as they can identify a food source that was more likely to have been eaten by cases than by controls; however, additional laboratory and/or traceback information is required to definitively identify the source.

This investigation also highlights the need to revisit strategies for effective investigation of locally acquired cyclosporiasis. Collecting good food exposure data and purchase information from cases at the time of initial case interviewing is important but challenging given the long incubation period and limited shelf life of produce. The lack of routinely available laboratory methods to type *Cyclospora* is another major investigative challenge. One possible solution would be to focus efforts on identifying and investigating event- or premise-based clusters where cases are more likely to have contracted their illness from one contaminated food product. Further discussion with produce-exporting countries where *Cyclospora* is endemic might help to address prevention and control measures.

Conclusion

Results from this outbreak investigation have contributed to the understanding of this disease in Canada and demonstrated that a control bank can be an effective tool for conducting case–control studies as part of public health investigations.

Authors' statement

VM — Investigation, methodology, writing (original draft and review & editing)
KM and MG — Investigation, methodology, writing (review & editing)
AFH, JH, LH and SD — Investigation, writing (review & editing)

Conflict of interest

None.

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Molecular surveillance of hepatitis C virus genotypes identifies the emergence of a genotype 4d lineage among men in Quebec, 2001–2017

DG Murphy[1]*, R Dion[1,2], M Simard[3], ML Vachon[4], V Martel-Laferrière[5], B Serhir[1], J Longtin[1]

Abstract

Background: Molecular phylogenetics are generally used to confirm hepatitis C virus (HCV) transmission events. In addition, the Laboratoire de santé publique du Québec (LSPQ) has been using molecular phylogenetics for surveillance of HCV genotyping since November 2001.

Objectives: To describe the emergence of a specific lineage of HCV genotype 4d (G4d) and its characteristics using molecular phylogenetics as a surveillance tool for identifying HCV strain clustering.

Methods: The LSPQ prospectively applied Sanger sequencing and phylogenetic analysis to determine the HCV genotype on samples collected from November 2001 to December 2017. When a major G4d cluster was identified, demographic information, HIV-infection status and syphilis test results were analyzed.

Results: Phylogenetic analyses performed on approximately 22,000 cases identified 122 G4d cases. One major G4d cluster composed of 37 cases was singled out. Two cases were identified in 2010, 10 from 2011–2014 and 25 from 2015–2017. Cases in the cluster were concentrated in two urban health regions. Compared to the other G4d cases, cluster cases were all male ($p<0.001$) and more likely to be HIV-positive (adjusted risk ratio: 4.4; 95% confidence interval: 2.5–7.9). A positive syphilis test result was observed for 27 (73%) of the cluster cases. The sequences in this cluster and of four outlier cases were located on the same monophyletic lineage as G4d sequences reported in HIV-positive men who have sex with men (MSM) in Europe.

Conclusion: Molecular phylogenetics enabled the identification and surveillance of ongoing transmission of a specific HCV G4d lineage in HIV-positive and HIV-negative men in Quebec and its cross-continental spread. This information can orient intervention strategies to avoid transmission of HCV in MSM.

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Keywords: HCV, genotype, G4d, surveillance, phylogenetic analyses, cluster, molecular epidemiology, men who have sex with men, MSM

Introduction

Hepatitis C virus (HCV) affects 70 million people worldwide and is a major public health concern. In Canada, nearly 250,000 persons, or 0.7% of the population, are estimated to be chronically infected with HCV and up to 44% of this population may be unaware of their status (1). In Quebec, 1,027 cases of HCV were reported in 2017 (incidence rate [IR] of newly reported cases of 12.2 per 100,000 population), with a projection estimate of 1,312 cases (IR of 15.5 per 100,000 population) for 2018 (2).

Based on prospective cohorts such as *SurvUDI* network and the *Engage* study, people who inject drugs and men who have sex with men (MSM) are disproportionally affected by HCV infection compared to the general population (2,3). Chronic HCV infection, if untreated, can result in fibrosis, cirrhosis, liver failure and hepatocellular carcinoma. Early identification of HCV infection followed by treatment is crucial in reducing HCV transmission, associated morbidity and mortality and health care costs (4).

HCV is mostly transmitted by the parenteral route, the most common risk factors being injection drug use and blood or blood product transfusion (prior to the introduction of blood donor screening). Sexual transmission of HCV is less common, but possible. Recent data indicates an increase in HCV prevalence among MSM, especially among those coinfected with HIV (5). High-risk behaviours, including unprotected sex, have been identified as determinants for HCV transmission. Ulcerative sexually transmitted infections, such as syphilis, has also been associated with increased risk of HCV acquisition in MSM (6,7).

HCV is currently classified into eight genotypes (8). Genotypes are further divided into subtypes, of which 89 have been confirmed (8). At the Laboratoire de santé publique du Québec (LSPQ), Institut national de santé publique du Québec, HCV genotyping has been performed routinely since November 2001 for patient management, as genotyping helps to guide antiviral treatment of chronic infection.

Genotyping performed by sequencing analysis not only informs optimal treatment choice, it can also be a powerful molecular surveillance tool for identification of circulating virus strains. Although molecular phylogenetics cannot determine the time of infection acquisition, it can detect transmission clusters. The laboratory can then notify public health authorities that a particular HCV strain is spreading in the population (9,10).

Molecular phylogenetics has not been used extensively for ongoing prospective surveillance, but studies have shown it to be a potentially useful tool. For example, nucleic acid sequencing and phylogenetic analysis have been used to identify and confirm HCV transmission events in the health care setting and to characterize transmission dynamics in the community (11–18). Phylogenetic strain clustering has also been used to investigate HCV transmission networks among HIV-infected MSM (19,20). A recent study supported the feasibility of applying nucleic acid sequencing and phylogenetics to identify recent HCV transmission clusters in individuals with unknown histories of transmission (21).

The objective of this article is to describe the use of molecular sequencing and phylogenetics as a tool for prospective surveillance of HCV transmission at the population level. Namely, we show how this approach identified the emergence and ongoing transmission of HCV genotype 4d (G4d) among HIV-positive and HIV-negative men in Quebec and how we were able to identify its source.

Methods

Study population

In Quebec, HCV genotyping is conducted at the LSPQ. Samples submitted for routine HCV genotyping from November 13, 2001 to December 31, 2017 were included in this study. Samples were submitted from hospital laboratories and public or private clinics in Quebec. Data on the G4d cases originated from the LSPQ's laboratory information system and included basic demographic information (age, sex, health region of residence), date of specimen collection and test results for HIV and syphilis infections. These were retrieved from the information system using patients' unique identifier numbers. Data on exposures or risk factors were not collected. Genotype results are not routinely reported to the public health regional authorities.

Genotyping and nucleotide sequence analysis

The HCV genotype of each sample was assessed as it was received. Most samples were received within 30 days of blood collection. Viral ribonucleic acid (RNA) extraction from serum or plasma as well as reverse transcription polymerase chain reaction (RT-PCR), DNA sequencing and genotyping based on nonstructural protein 5B (NS5B) sequences were performed as previously described (22). Quebec's G4d sequences were compared to sequences previously reported in HIV-infected MSM in the Netherlands and France (23–25). To compare Quebec's G4d sequences with those reported in the van de Laar et al. study (20) for participants from England, France, Germany and the Netherlands, the corresponding NS5B nucleotide sequence was obtained for a subset of Quebec's G4d isolates by use of reverse primer DM503 (5'-CCACGCTCTCAACGGTGGTAC-3) and forward primer DM101 to generate a 805 base pair sequence fragment (22). These were selected on the basis of sample availability. Phylogenies were estimated by the neighbour-joining method according to the maximum composite likelihood model of nucleotide substitution implemented in Molecular Evolutionary Genetic Analysis (MEGA) software version 6 (26). As many as 1,000 bootstrap replicates were performed to evaluate the robustness of the phylogeny. Sequences were considered to be part of a cluster if the following three criteria were met: showed less than 3% nucleotide difference; displayed a bootstrap support value of greater than 70%; and consisted of at least three cases. The closeness of cases in terms of time and space was not included in this definition. G4d sequences for isolates reported in this study were submitted to GenBank and can be retrieved under accession numbers MK950000 to MK950149. NS5B sequences for isolates 3_QC55 (EF116095), 10_QC307 (EF116147), and 27_QC382 (FJ462437) were submitted previously to GenBank.

Statistical analysis

Descriptive analysis of the data was conducted using Epi Info software version 7.2.2.6 (27) and statistical package SAS version 9.3 (28). This analysis was based on specimen collection dates, grouped as quarterly periods. For univariate analysis, statistical comparisons of categorical variables (age group, sex, health region, G4d HCV cluster and HIV infection status) were performed using bilateral Mantel–Haenszel chi square test and modified Poisson regression with a robust error variance sandwich estimation (29). To assess their link with cluster A outcome, the multivariate analysis using robust Poisson regression included the following independent variables in the model: HIV infection (yes versus no or unknown) and age group (50 years or older vs younger than 50 years). Given that none of the cluster A cases were female, this analysis was restricted to males. Statistically significant levels for confidence intervals (CI) of risk ratio (RR) estimates and bilateral *p* values were set at 5%.

Results

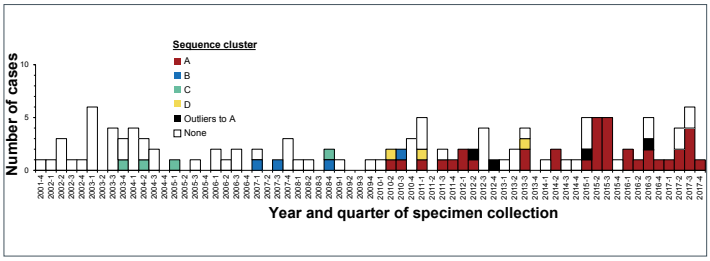
From November 13, 2001 to December 31, 2017, HCV genotypes were determined from a total of approximately 22,000 cases. G1 was the most prevalent genotype (59.7%), followed by G3 (25.7%), G2 (8.6%), G4 (3.8%), G6 (1.6%), G5 (0.6%) and G7 (0.01% [three cases only]). Phylogenetic analyses of NS5B sequences revealed notable clusters mainly among genotypes 1a, 1b, 2b, 3a and 4d. One major G4d cluster composed of 37 cases was singled out and is described in this study.

Overall, G4 was found in 834 cases. Five G4 cases displayed low levels of viremia and the genotype was determined based on the 5'UTR (5' untranslated region) sequence, which is too conserved for subtype determination. G4d was found in 122 (14.7%) of 829 G4 cases for which the subtype was available.

Strain clustering of G4d cases

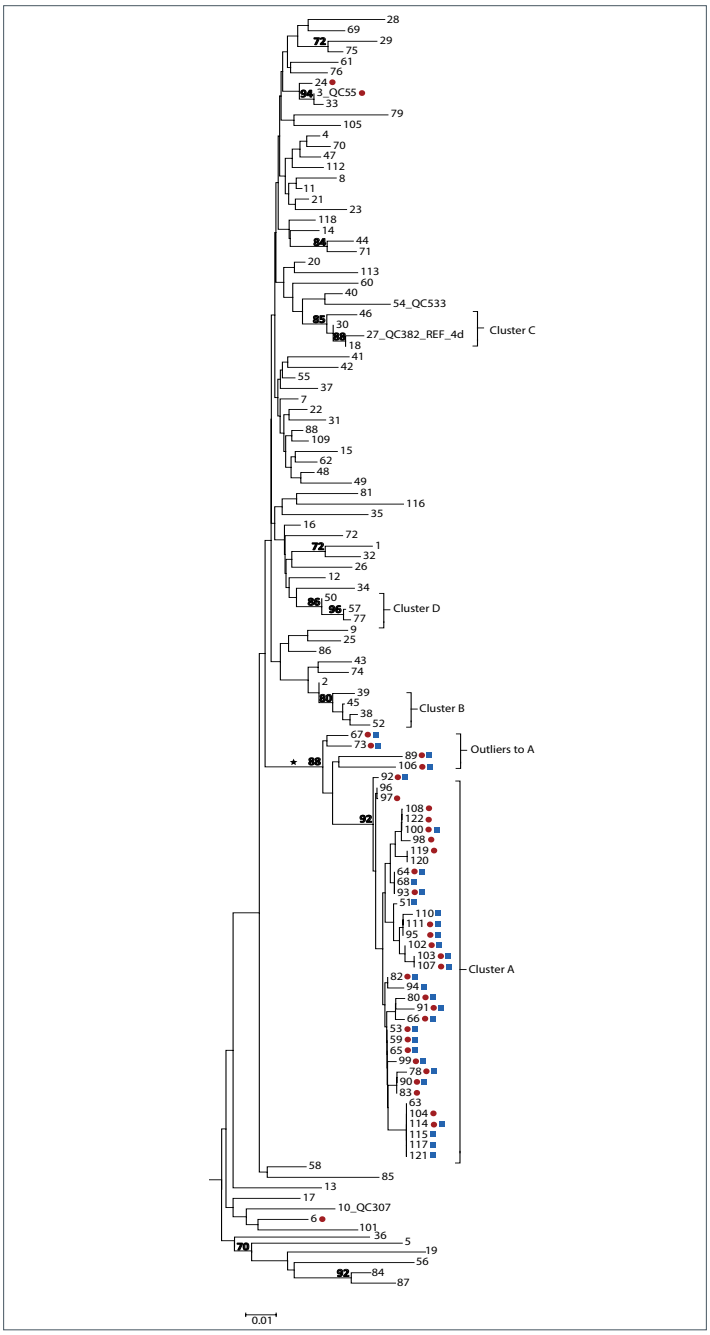
The first G4d case was recorded in December 2001 (**Figure 1**). From 2002 to 2014, an average of six cases per year was observed. In 2015, 16 cases were noted. This increase continued throughout 2016 and 2017. A phylogenetic tree was constructed based on NS5B sequences for the 122 G4d cases and analyzed for strain clustering. Four G4d clusters (named A to D) were identified (Figure 1 and **Figure 2**). Cluster A comprised 37 cases, clusters B and C each comprised four cases, and cluster D comprised three cases. G4d cluster A cases first appeared in the

Figure 1: Number of hepatitis C virus genotype 4d cases by year and quarter of specimen collection and sequence clusters, November 2001 to December 2017, Quebec, Canada



Source: Laboratoire de santé publique du Québec (December 2018)

Figure 2: Phylogenetic tree of genotype 4d NS5B hepatitis C virus isolate sequences, Quebec, Canada, November 2001 to December 2017 (n=122)



Abbreviation: NS5B, nonstructural protein 5B
Legend:
Tree inferred from NS5B sequences corresponding to nucleotide positions 8276-8615
Red circles, HIV-1 positive; blue squares, treponemal test positive
Numbers at nodes represent the percent bootstrap values (only values greater than 70% are shown on nodes of non-identical sequences)
The cross-continental G4d monophyletic lineage is indicated by an asterisk
Source: Laboratoire de santé publique du Québec (December 2018)

second quarter of 2010. Cases within each of clusters B, C and D were scattered in time (Figure 1). An increase in cluster A cases was observed from the second quarter of 2015 to the fourth quarter of 2017, for which 24 of 32 (75%) G4d cases were part of this cluster.

Four cases (designated as outliers to cluster A) that localized on the same monophyletic lineage as cluster A cases were also identified. However, these were excluded as they displayed greater than 3% nucleotide differences from cluster A strains (Figure 1 and Figure 2). Outliers to cluster A cases were identified during the same period as cluster A cases. Sequences of cluster A and of the four outliers localized on the same monophyletic lineage as G4d sequences were reported in HIV-positive MSM in England, France, Germany and the Netherlands (Figures A1 and A2 in **Appendix 1**) (20,23–25).

Demographics and other sexually transmitted infections

On univariate analysis, cluster A cases, compared to the other G4d cases, were more likely to be male (100% vs 62%; RR: undefined; $p<0.001$), HIV-positive (73% vs 8%; RR: 6.99; 95% CI: 3.80–12.84; $p<0.001$), and 50 years or older (60% vs 34%; RR: 2.04; 95% CI: 1.18–3.54; $p=0.01$). In multivariate analysis restricted to male cases, age group (adjusted RR [aRR]: 1.66; 95% CI: 1.11–2.50; $p=0.02$) and HIV infection (aRR: 4.43; 95% CI: 2.49–7.88; $p<0.001$) remained significantly associated with the cluster A outcome. Of cluster A cases, 27 (73%) had a history of positive treponemal test results for syphilis and 20 (57%) were both HIV-positive and had a history of positive treponemal test results.

Cluster A cases resided in seven of the 18 health regions in Quebec, but were concentrated in two nonadjacent and predominantly urban regions. These two regions accounted for 78.3% of cluster A cases (**Table 1**). Of note, in one of the urban regions (health region Y), 14 of 24 (58.3%) observed G4d cases were part of cluster A. Although the IR of newly notified cases of HCV in health region Y was fourfold lower than in health region X from 2013–2017, an equivalent number of G4d cluster A cases was observed in these two health regions (14 vs 15). The cases not in cluster A resided in 14 health regions, of which the one with the largest population of the province accounted for 49.4% of cases.

Discussion

In this study, we found that HCV molecular phylogenetics identified the transmission of a specific lineage (cluster A) of HCV G4d among men in Quebec; this would otherwise have remained undocumented. The G4d cluster A strains were found to be concentrated in two urban health regions (X and Y) and localized on the same monophyletic lineage as G4d sequences reported in HIV-positive MSM in England, France, Germany and the Netherlands (20,23–25) and later in Spain (30). Although it is likely that this particular G4d cluster A strain was introduced to Canada from Europe, the origin of this strain in Quebec remains unknown.

These findings suggest that high-risk sexual behaviour may be the mode of spread of G4d among the cluster A cases. The international transmission network of this strain in HIV-infected MSM are not injection drug users. There was also a high prevalence of positive treponemal test results. Syphilis is considered as a marker of high-risk sexual behaviour (31). Ulcerative sexually transmitted infections, such as syphilis, have been associated with increased risk of sexual HCV acquisition in MSM (6,7).

In 2015, the emergence of this cluster was brought to the attention of provincial public health authorities and the two regional public health authorities most affected. Since sexually active HIV-negative MSM are also at risk of HCV infection, annual HCV testing is recommended for MSM who engage in HIV preexposure prophylaxis (32).

Table 1: Number, proportion and mean incidence rate of hepatitis C virus and number and proportion of hepatitis C virus genotype 4d cases among cluster A and others, by health regions, Quebec

Health region	Population in 2015 (n)[a]	Mean annual HCV cases, 2013–2017 (2)			HCV G4d cases from November 2001 to December 2017[b]					
		n	%	IR[c]	In cluster A		Not in cluster A		Total	
					n	%	n	%	n	%
X	1,992,106	399	36.6	20.0	15	40.5	42	49.4	57	46.7
Y	736,787	92	8.4	12.5	14	37.8	10	11.8	24	19.7
Others	5,562,462	600	55.0	10.8	8	21.6	33	38.8	41	33.6
Total	8,291,355	1,091	100.0	13.2	37	100.0	85	100.0	122	100.0

Abbreviations: G4d, genotype 4d; HCV, hepatitis C virus; IR, incidence rate
[a] Ministère de la santé et des services sociaux du Québec, demographic estimates and projections, March 2018
[b] Source: Laboratoire de santé publique du Québec (December, 2018)
[c] Mean incidence rate of notified cases per 100,000 persons per year

Though most cases in cluster A were coinfected with HIV, 27% (10/37) had no laboratory evidence of HIV infection. This indicates likely transmission from HIV-positive to HIV-negative men or vice versa. HCV strain clustering, including G4d, among HIV-positive and HIV-negative MSM has been observed in France and the Netherlands in study participants enrolled in HIV preexposure prophylaxis programs (24,33).

Strengths and limitations

The main strength of this study is the high number of HCV-infected cases (about 22,000) for whom uniform sequencing results were available through the study period. An added strength was that the genotyping was performed in the same central reference laboratory. The estimated cumulative number of HCV chronic infections in Quebec reached 42,000 in 2017; nearby 75% can be assumed to be viremic (2). Thus, the number of individuals included in this study represents a significant proportion of cases who have been diagnosed and are viremic. Nevertheless, G4d cluster A cases are likely to be underestimated as some may not have been diagnosed; in addition, genotyping tests may not have been requested for all cases. Even with this underestimation, the genotyping data was probably unbiased given that it was not directed at specific subgroups of the population.

A limitation of this analysis is that there is no strict definition of strain clustering, as it varies depending on the region of the genome analyzed and the study population. In addition, the rate of HCV evolution can vary over time, based on the individual, and may impact clustering of viral strains. Also, the use of longer HCV sequences could have increased the accuracy of cluster identification (34). This study did not include data about determinants, exposures, sexual orientation or epidemiologic links between cases, and could not distinguish the mode of transmission of infection; as such, it can only provide indirect clues of HCV propagation among Quebecois men, most probably MSM.

Conclusion

Molecular phylogenetic-based surveillance revealed an ongoing transmission in Quebec of a specific cluster of HCV G4d. This cluster localized on the same monophyletic lineage as G4d sequences reported in HIV-infected MSM in several countries in Europe, indicating a cross-continental spread of this specific lineage. Phylogenetic analysis results coupled with basic demographic data produces an epidemiologic profile of HCV cases that can orient preventive interventions to avoid HCV transmission among sexually active HIV-positive and HIV-negative MSM.

Authors' statement

DGM — Conceptualization, methodology, software, data collection and conservation, validation, official analysis, writing of the first draft, display, supervision, project administration
RD — Conceptualization, methodology, software, official analysis, writing the first draft, review and revision of the final version, display
MS — Conceptualization, methodology, software, official analysis, review and revision of the final version
MLV — Clinical expertise, review and revision of the final version
VML — Clinical expertise, review and revision of the final version
BS — Data collection, review and revision of the final version
JL — Project administration, review and revision of the final version, supervision

Conflict of interest

None.

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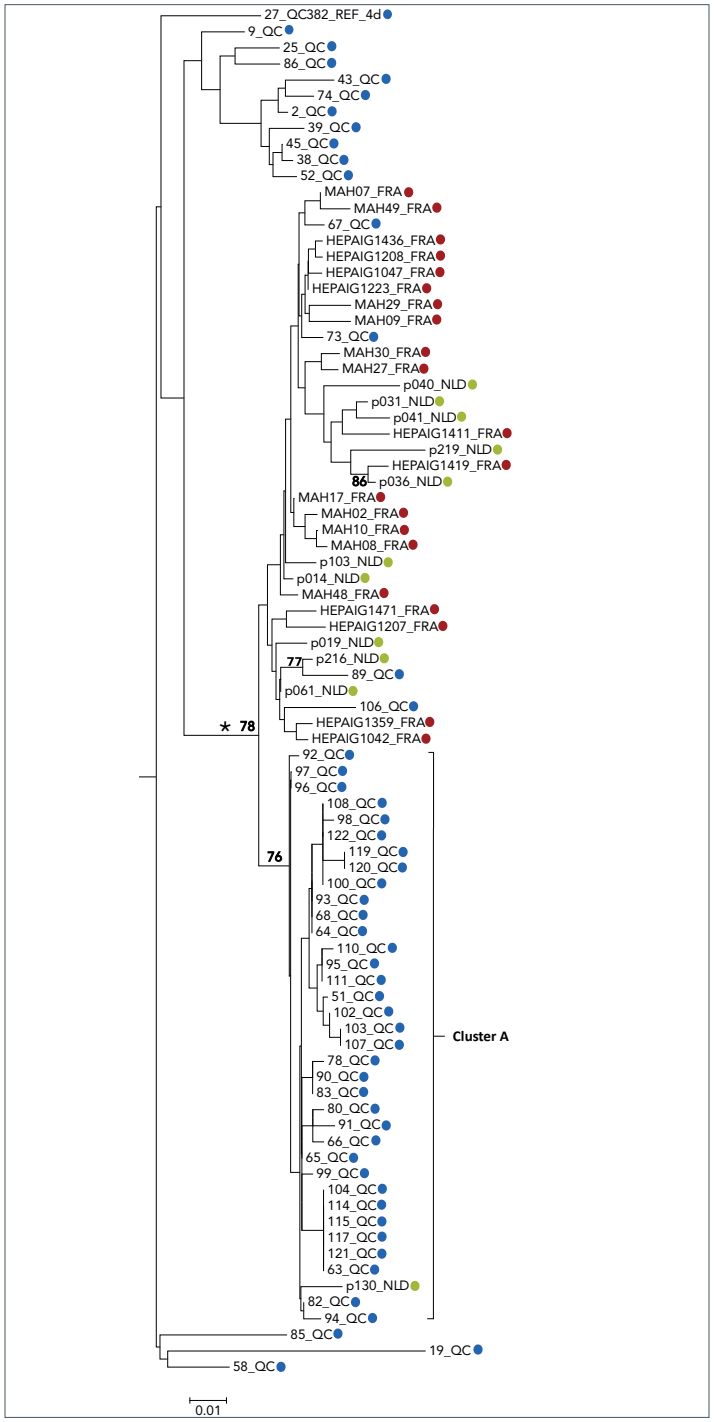
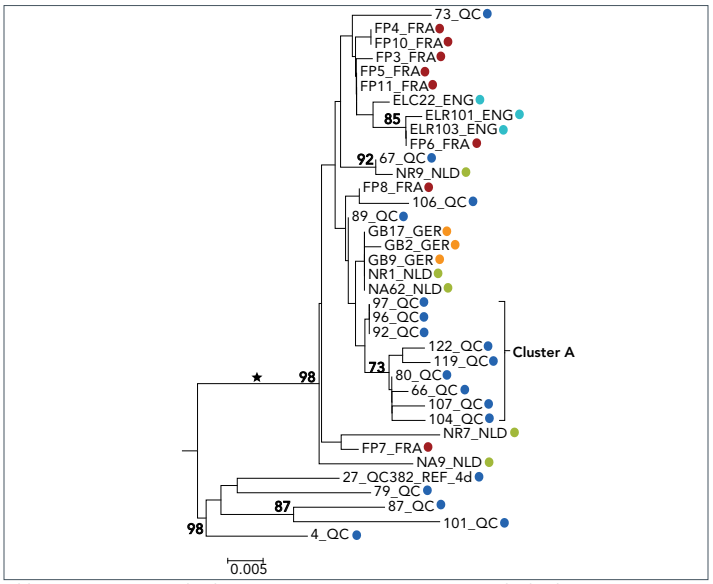
Appendix: Supplementary data

Figure A1: Phylogenetic tree of genotype 4d NS5B hepatitis C virus isolate sequences: cases in Quebec compared to cases in France and the Netherlands



Abbreviations: FRA, France; NLD, the Netherlands; NS5B, nonstructural protein 5B; QC, Quebec
Legend:
Tree inferred from NS5B sequences corresponding to nucleotide positions 8276-8615
Blue, Quebec; red, France; green, the Netherlands
Numbers at nodes represent the percent bootstrap values (only values greater than 70% are shown on nodes of non-identical sequences)
The cross-continental G4d monophyletic lineage is indicated by an asterisk
Source: Laboratoire de santé publique du Québec, December 2018 and references 23–25

Figure A2: Phylogenetic tree of genotype 4d NS5B hepatitis C virus isolate sequences: cases in Quebec compared to cases in England, France, Germany and the Netherlands



Abbreviations: ENG, England; FRA, France; GER, Germany; NLD, Netherlands; NS5B, nonstructural protein 5B; QC, Quebec
Legend:
Tree inferred from NS5B sequences corresponding to nucleotide positions 8547-8982
Blue, Quebec; red, France; green, the Netherlands; turquoise England; orange Germany
Numbers at nodes represent the percent bootstrap values (only values greater than 70% are shown on nodes of non-identical sequences)
The cross-continental G4d monophyletic lineage is indicated by an asterisk
Source: Laboratoire de santé publique du Québec, December 2018 and reference 20

Shiga toxin–producing *Escherichia coli* in British Columbia, 2011–2017: Analysis to inform exclusion guidelines

K Noftall[1,2], M Taylor[1], L Hoang[3,4], E Galanis[1,2]

Abstract

Background: Shiga toxin–producing *Escherichia coli* (STEC) can cause severe illness including bloody diarrhea and hemolytic-uremic syndrome (HUS) through the production of Shiga toxins 1 (Stx1) and 2 (Stx2). *E. coli* O157:H7 was the most common serotype detected in the 1980s to 1990s, but improvements in laboratory methods have led to increased detection of non-O157 STEC. Non-O157 STEC producing only Stx1 tend to cause milder clinical illness. Exclusion guidelines restrict return to high-risk work or settings for STEC cases, but most do not differentiate between STEC serogroups and Stx type.

Objective: To analyze British Columbia (BC) laboratory and surveillance data to inform the BC STEC exclusion guideline.

Methods: For all STEC cases reported in BC in 2011–2017, laboratory and epidemiological data were obtained through provincial laboratory and reportable disease electronic systems, respectively. Incidence was measured for all STEC combined as well as by serogroup. Associations were measured between serogroups, Stx types and clinical outcomes.

Results: Over the seven year period, 984 cases of STEC were reported. A decrease in O157 incidence was observed, while non-O157 rates increased. The O157 serogroup was significantly associated with Stx2. Significant associations were observed between Stx2 and bloody diarrhea, hospitalization and HUS.

Conclusion: The epidemiology of STEC has changed in BC as laboratories increasingly distinguish between O157 and non-O157 cases and identify Stx type. It appears that non-O157 cases with Stx1 are less severe than O157 cases with Stx2. The BC STEC exclusion guidelines were updated as a result of this analysis.

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Keywords: Shiga toxin, *Escherichia coli*, guidelines, surveillance, STEC

Introduction

Escherichia coli has long been known to be part of the normal flora of the gastrointestinal tract. Some *E. coli* strains evolved to cause human illness by acquiring virulence factors such as Shiga toxin. These organisms have been renamed Shiga toxin–producing *E. coli* (STEC) (1). The best known STEC serotype, *E. coli* O157:H7, was first detected in the 1980s in patients with serious illnesses including hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (2,3). In the 1990s, once laboratories started to routinely test for *E. coli* O157:H7, it became recognized as the cause of large and serious outbreaks mainly associated with undercooked beef (4,5).

In the early 2000s, laboratories began to implement methods to detect other STEC serotypes (6,7). This led to the recognition that non-O157 STEC can contaminate a wide variety of sources and cause large outbreaks (8,9). However, non-O157 STEC remain underreported because many frontline laboratories

do not yet routinely test for them (10). Nonetheless, by 2016, approximately 35% of all STEC infections reported to the Canadian National Enteric Surveillance Program were caused by non-O157 serotypes (11).

STEC *stx* genes produce Shiga toxins 1 (Stx1) and 2 (Stx2). Stx2 is associated with severe illness and HUS (8,12–20). Furthermore, Stx2 is associated with STEC O157, which explains this serogroup's higher pathogenicity (17,21,22). A polymerase chain reaction (PCR) test was implemented at the British Columbia Centre for Disease Control Public Health Laboratory (BCCDC PHL) in 2013 to detect and differentiate between *stx* 1 and 2 genes.

In order to protect the population from severe infection, most public health authorities exclude people infected with STEC from working or attending certain high-risk settings (23,24). For example, the 2006 BCCDC guidelines stated that a person diagnosed with STEC who works as a food handler, health care worker or child care worker or who attends a child care facility should be excluded from that setting until they have provided two negative stool samples (25).

The increasing detection of non-O157 STEC and their association with Stx1 and less severe disease led us to question the appropriateness of exclusion guidelines that do not differentiate between serogroups and Stx types. A review of existing guidelines demonstrated that four regions had recently updated their recommendations to stratify exclusion from workplaces and daycares by serogroup and Stx type (26–29).

The purpose of this work was to analyze British Columbia (BC) laboratory and surveillance data to inform the BC STEC exclusion guideline.

Methods

Study sample

This study included all confirmed cases of STEC who were BC residents and reported in the provincial electronic public health information system between January 1, 2011 and December 31, 2017 (30). For these cases, all STEC culture, serogroup and PCR *stx* results were accessed from the BCCDC PHL information system.

Laboratory data

In BC, stool specimens may be processed at a private laboratory, hospital laboratory or the BCCDC PHL. At private and hospital laboratories, STEC are identified using culture or molecular methods. STEC isolates that are Stx positive or O157, as well as specimens that are visibly bloody or that came from a case diagnosed with HUS, are sent to the BCCDC PHL for further testing and serotyping. Until 2013, Stx was detected using a Vero cell assay at the BCCDC PHL; since 2013, *stx* 1 and 2 genes have been detected by PCR from stool samples and from suspected STEC isolates recovered from *stx*-positive stool samples from private and hospital laboratories. Positive *stx* isolates are serotyped for O157 antigen, H7 antigen and an O-Typer PCR covering the six most common serogroups seen in BC: O26, O45, O103, O111, O121 and O145. Any *stx*-positive isolate that cannot be serotyped is sent to the National Microbiology Laboratory for identification.

Variables included from BCCDC PHL data were Stx type and serogroup. If Vero cell assay was used, the Stx result was reported as positive; when PCR was performed, the *stx* gene was recorded. When multiple laboratory test results were available for individual cases, the most specific serotype result was included.

Surveillance data

STEC cases are reportable in BC and are interviewed by public health professionals using a standard surveillance form (30,31). All health authorities report STEC cases provincially through the BC provincial electronic public health information system. Self-reported data on demographics, clinical symptoms, hospitalization and HUS status were extracted from this system. Laboratory data may also be entered into this system as part of routine surveillance by the health authority.

Hospitalization status and exclusion information were only available for cases from 2015–2017. Clinical data were available from four of five health authorities. Cases were considered to have a symptom or outcome if "yes" was recorded for that data field. If no laboratory results were available from BCCDC PHL, the laboratory information reported by the health authorities into the public health information system was used.

Analysis

BCCDC STEC surveillance data and BCCDC PHL STEC lab data from 2011 to 2017 were linked together by a common case identifier. Incidence rates were calculated using the BC population data (32). Differences in STEC incidence rates between females and males were conducted using the Wilcoxon rank sum test. Clinical information was compared by serogroups and by Stx type for all symptoms as well as for HUS and hospitalization using Fisher exact test. An alpha level of 0.05 was used for significance, and all statistical tests were conducted using R statistical computing program (33). When exclusion information was available, excluded cases were categorized by serogroup and Stx type.

Results

Between 2011 and 2017, 984 cases of STEC were reported in BC, with an average of 141 cases per year (range: 108–184). The overall STEC incidence during this time period was 3.0 cases per 100,000. Outbreaks occurred in 2013, 2016 and 2017. There were more female (57.4%) than male (42.6%) cases, but there was no significant difference in rates by sex for all STEC cases or by serogroups (data not shown). The median age was 31 years (range <1–113), and the highest incidence was in 1–9 year olds at 4.9 cases per 100,000.

In total, 58 serogroups were identified. Nearly 10% of samples were culture negative and *stx* positive only (**Table 1**). The six most common serogroups were O157, O121, O26, O103, O117 and O111. The most reported serogroup was O157 for every year except 2017, when O121 cases were more common.

Overall, O157 rates have declined from 1.1 to 0.5 per 100,000 from 2011 to 2017 (**Figure 1**). Non-O157 rates have increased greatly from 0.6 to 2.4 per 100,000 between 2011 and 2017.

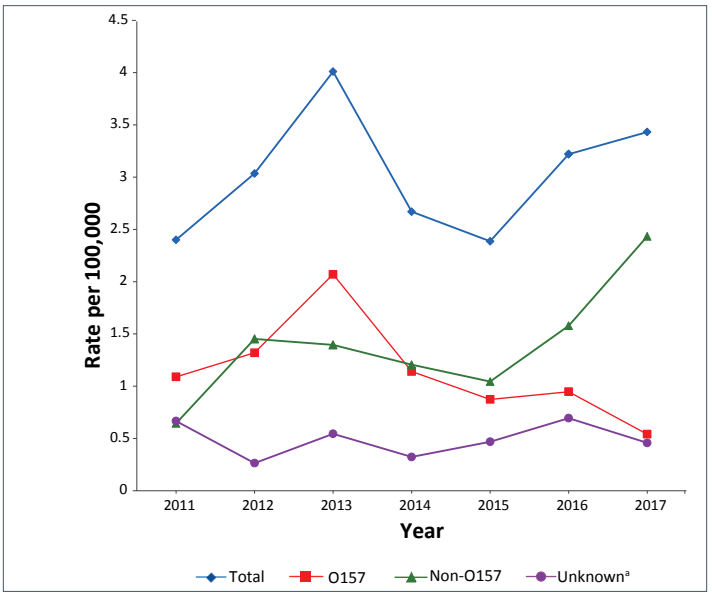
Table 1: Serogroup and Shiga toxin results for Shiga toxin–producing *Escherichia coli* cases, British Columbia, 2011–2017 (N=984)

Serogroup	N	(%)
O157	369	(37.5)
O121	102	(10.4)
O26	86	(8.7)
O103	44	(4.5)
O117	38	(3.9)
O111	34	(3.5)
Other serogroups	152	(15.4)
Stx positive only	93	(9.5)
Unknown[a]	66	(6.7)
Shiga toxin	**N**	**(%)**
Stx positive[b]	111	(11.3)
Stx1	270	(27.4)
Stx1 and Stx2	239	(24.3)
Stx2	274	(27.8)
Stx unknown[c]	90	(9.1)

Abbreviations: Stx, Shiga toxin; Stx1, Shiga toxin 1; Stx2, Shiga toxin 2
[a] Cases where no laboratory results were available at the British Columbia Public Health Laboratory. Cases were reported solely by public health authorities
[b] Cases where Shiga toxin was detected by Vero cell assay and not a polymerase chain reaction (PCR)
[c] Includes cases reported solely by public health authorities (N=81) and those reported by the Public Health Laboratory for which Stx type was not available for epidemiological analysis (N=9)

The presence of Stx was confirmed in 894 cases (90.1%; Table 1). For 111 cases (11.3%), Stx was detected by Vero cell assay only, and specific Stx type was not available. Of the 783 cases where the *stx* gene was identified by PCR, 270 (34.5%) were positive for *stx*1 only, 274 (35.0%) were positive for *stx*2 only, and 239 (30.5%) were from STEC that contained both *stx*1 and *stx*2. Therefore, 65.5% of cases where the *stx* gene was detected tested positive for *stx*2 (alone or in combination). When both serogroup and *stx* gene were identified (N=711), 97.7% of O157 STEC contained *stx*2 alone or in combination compared to 42.9% of non-O157 serogroups [odd ratio (OR) = 56.5; $p \leq 0.001$].

Figure 1: Shiga toxin–producing *Escherichia coli* incidence by serogroup, British Columbia, 2011–2017 (N=984)



[a] Cases who are Shiga toxin–positive cases only and cases reported solely by public health authorities without laboratory information from the Public Health Laboratory

Clinical information was available for 594 cases (60.4%). The most commonly reported symptoms were diarrhea (90.2%), abdominal discomfort (70.2%), bloody diarrhea (56.6%), vomiting (24.6%), fever (21.4%) and fatigue (13.6%). Eighteen cases (3.0%) were recorded as having HUS at the time of the public health interview. Cases with *E. coli* O157 infection were more likely to have bloody diarrhea, fatigue and hospitalization than non-O157 cases (**Table 2**). Although a larger proportion of O157 cases (4.9%) had HUS than non-O157 cases (1.9%), this was not statistically significant. Cases with *stx*2 were significantly more likely to have bloody diarrhea and HUS and be admitted to hospital than cases with *stx*1 only (Table 2).

Exclusion information was available for 276 (64%) cases between 2015 and 2017. Forty-three cases (16%) had been excluded from high-risk occupations or settings. Ten of the cases were positive for O157 (23.3%), 28 were positive for a non-O157 STEC (65.1%) and five had an unknown serogroup (11.6%). Twenty-one cases (48.8%) had STEC with *stx*2 present, 20 (46.5%) had STEC with *stx*1 only and two (4.7%) had STEC with indeterminate *stx* PCR results. All O157 STEC cases were *stx*2 positive.

Table 2: Clinical severity by *Escherichia coli* serogroup and Shiga toxin type among Shiga toxin–producing *Escherichia coli* cases, British Columbia, 2011–2017[a] (N=594)

Clinical outcome	Serogroup					Shiga toxin type				
	O157		Non-O157		*p*-value	Stx2 present		Stx1 only		*p*-value
	N	(%)	N	(%)		N	(%)	N	(%)	
Abdominal discomfort	177	(72.5)	189	(70.3)	0.625	262	(73.8)	106	(69.3)	0.330
Diarrhea	221	(90.5)	250	(92.9)	0.339	321	(90.4)	138	(90.2)	1.000
Bloody diarrhea	168	(68.9)	136	(50.6)	<0.001	231	(65.1)	69	(45.1)	<0.001
Fatigue	46	(18.9)	25	(9.3)	0.002	53	(14.9)	17	(11.1)	0.326
Fever	57	(23.4)	50	(18.6)	0.193	78	(22.0)	27	(17.6)	0.285
Vomiting	63	(25.8)	64	(23.8)	0.610	85	(23.9)	34	(22.2)	0.733
HUS	12	(4.9)	5	(1.9)	0.081	15	(4.2)	1	(0.7)	0.048
Hospitalised[b]	29	(35.4)	25	(16.1)	0.001	52	(31.7)	13	(13.8)	0.002

Abbreviations: HUS, hemolytic-uremic syndrome; Stx1, Shiga toxin 1; Stx2, Shiga toxin 2
[a] Information from cases in four of five health authorities; only cases with known serogroup or Shiga toxin type included
[b] Hospitalization status only available for cases from 2015 to 2017

Discussion

Analysis of laboratory and surveillance data in BC showed a shifting pattern in the distribution of STEC serogroups between 2011 and 2017 with the incidence of O157 declining and the incidence of non-O157 increasing, particularly between 2015 and 2017. This is consistent with the decline in the incidence of O157 that has been observed across Canada (34) and the United States (6,8,22,35,36). The decrease in O157 in Canada may be due to sanitary improvements in beef processing as well as better food safety education to consumers (34). The increase in incidence of non-O157 in BC and elsewhere may be due to the increase in use of laboratory methods enabling the detection of non-O157 strains (6,36).

A strong association was seen between the O157 serogroup and Stx2 with nearly 98% of O157 cases being positive for Stx2, consistent with earlier studies (15,16,21,22). Also observed were the associations between O157 STEC cases and severe clinical outcomes such as hemorrhagic colitis and hospitalization (17,22,37). There was a significant relationship between *stx*2 and HUS, bloody diarrhea and hospitalization. Stx variants (e.g. *stx*2d), which have been shown to be more specific predictors of severe illness than Stx type, are not yet available in BC (13,15,16,18–20,38).

This analysis was conducted to inform the BC STEC exclusion guideline. Consistent with our findings and other recently updated guidelines stratifying exclusion by serogroup and Stx (26–29), the new BC guideline now reflects the evidence that STEC producing Stx1 only leads to less severe illness (39). Based on the revised guideline whereby non-O157 *stx*1 only cases without severe clinical symptoms could return to high-risk work or settings when symptom-free, 16 excluded cases (37.2%) between 2015 and 2017 would have had their exclusion lifted earlier. Assuming the median duration of symptoms is seven days and the median duration of shedding is 20 days (40,41), this could have resulted in avoiding up to 208 days of lost productivity for these individuals.

This study has a few limitations. Laboratory practice for Stx detection changed during the study period, so we were unable to differentiate Stx types between 2011 and 2013. Symptoms were self-reported and only available for four of the five health authorities in the province. Despite this, the proportion of cases with symptoms was similar to that observed in other studies (22,42). There is no reason to believe symptoms and outcomes for the same disease would be different for the one health authority from which these data were unavailable. The number of HUS cases was likely an underestimate given that HUS may not have developed at the time of the public health interview, which occurs one to two weeks after symptom onset (43). Nonetheless, a significant association with *stx*2 was observed, and this association may be even stronger with a higher number of HUS cases. Exclusion information was not available prior to 2015 and was not always recorded after that. Because of this, only a descriptive analysis was performed for excluded cases.

An evaluation of this guideline change in BC is recommended to ensure its impact was as initially intended. Furthermore, the guideline will need to be reviewed as laboratory methods for

STEC and Stx detection progress, such as by testing for specific Stx variants.

Conclusion

Data from BC is consistent with observations elsewhere that STEC bacteria cause a spectrum of illness and this is, at least in part, determined by Stx type. As a result of this analysis, the BC STEC exclusion guideline was updated in February 2019 to allow cases infected with non-O157 *stx*1 only STEC without severe clinical symptoms to return to high-risk settings when symptom-free.

Authors' Statement

KN — Analyzed the data and drafted the paper
MT — Conceptualized the study, interpreted the data and revised the paper
LH — Interpreted the data and revised the paper
EG — Conceptualized the study, interpreted the data, drafted certain sections and revised the paper

Conflict of interest

None.

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Surveillance of laboratory exposures to human pathogens and toxins: Canada 2018

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Abstract

Background: The Laboratory Incident Notification Canada (LINC) surveillance system monitors laboratory incidents reported under the *Human Pathogens and Toxins Act*. The year 2018 marks the third complete year of data.

Objective: To describe the laboratory exposure and laboratory-acquired infection incidents that occurred in Canada in 2018 compared to previous years, and then by sector, human pathogens and toxins involved, number of affected persons, incident type and root causes.

Methods: Laboratory incidents that occurred in 2018 were reported through the LINC system. The number of laboratory incidents, people exposed and laboratory-acquired infections were compared to previous years, then the incidents were analyzed by sector, human pathogen or toxin involved, the type of incident, people exposed, route of exposure and root causes. Microsoft Excel 2016 was used for descriptive analysis.

Results: In 2018, there were 89 exposure incidents to human pathogens and 235 people were exposed. There were five suspected and one confirmed laboratory-acquired infections. This was approximately twice the number of exposure incidents that were reported in 2017 (n=44) and 2016 (n=46). The highest number of exposure incidents occurred in the academic and hospital sectors, and the ratio of incidence to licences was the lowest in the private sector. The majority of incidents (n=50; 56%) involved Risk Group 2 human pathogens that were manipulated in a Containment Level 2 laboratory. Most exposures were related to sharps or procedures and the most common people exposed were laboratory technicians. Human interaction and standard operating procedures were the leading root causes.

Conclusion: Although overall the annual incidence of laboratory exposures in Canada remains relatively low, the incidence was higher in 2018 than in previous years. Whether this is a true increase in incidence or an increase in reporting is not known at this time as baseline estimates are still being established.

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Keywords: laboratory exposures, laboratory incidents, laboratory-acquired infections, human pathogens and toxins, surveillance, Laboratory Incident Notification Canada, Centre for Biosecurity

Introduction

Laboratory work with human pathogens and toxins involves risk of incidents that can cause exposures through accidental or deliberate release. These exposures can affect individuals directly involved in the incident or in proximity. They can potentially be a public health threat if community transmission occurs. Timely reporting of exposure incidents is critical for the prevention of potential outbreaks, as it allows rapid action in response to detected exposures.

In recent years, there has been growing public concern about the potential of a pandemic arising from laboratory-acquired infections as more countries are allowing gain-of-function studies, where researchers are increasing transmissibility and virulence of pathogens such as influenza virus (1,2). Having a strong biosafety and biosecurity regime is essential to address these concerns.

The Public Health Agency of Canada's Centre for Biosecurity is mandated to prevent, detect and respond to public health risks posed by the use of human pathogens and toxins in Canada. The Centre for Biosecurity oversees activities conducted under the *Human Pathogens and Toxins Act* (HPTA) and the *Human Pathogens and Toxins Regulations* (HPTR). In December 2015, the Centre for Biosecurity established a national surveillance system, the Laboratory Incident Notification Canada (LINC), for the mandatory reporting of laboratory incidents involving human pathogens and toxins. Facilities conducting controlled activities with human pathogens and toxins are required to be licensed. One licence can represent multiple containment zones, but a single licence cannot cover different risk groups (RGs). Containment zones can comprise typical laboratory working areas, vaccine production areas or even animal care facilities. Licensed facilities determine how to set up the framework of their licences based on these requirements. Upon registering for a licence, facilities self-identify the one sector that best represents them in terms of organizational structure. The sector choices are academic, hospital, private industry/business, public health or other government.

In accordance with the HPTA/HPTR, facilities conducting controlled activities with human pathogens and toxins must notify the Centre for Biosecurity at the Public Health Agency of Canada without delay of laboratory incidents involving pathogens and toxins at RG2 or higher levels (3). The Centre for Biosecurity provides a response without delay including timely follow-up. This could include biosafety advisories and other alerts regarding emerging trends detected and potential patterns of concern for continuous improvement of biosafety and biosecurity in Canada.

Notifications submitted to the LINC system can include exposure and nonexposure incidents. A laboratory incident can involve a potential or actual exposure to a biological agent, whether it causes a laboratory-acquired infection or not. A non-exposure incident can include inadvertent possession, production or release of a pathogen or toxin; a missing, lost or stolen pathogen or toxin; or a security-sensitive biological agent not received within 24 hours of expected arrival (4). In order to maximize the reporting of these incidents and ensure compliance with the HPTA, the Centre for Biosecurity conducts routine compliance promotion, monitoring and verification activities. It has also been reporting information on laboratory incidents annually to stimulate information sharing, increase awareness and promote reporting (5,6).

This study provides a descriptive summary of laboratory incidents that occurred in Canada in 2018, focussing on data of exposures and laboratory-acquired infections. The objective of this report is to briefly compare exposure incidents to the data from previous years and to describe laboratory exposures by sector, human pathogen and toxin involved, incident type, people exposed, route of exposure and root causes.

Methods

Data sources

The Biosecurity Portal is the "outward facing" portion of the LINC that facilitates notification and reporting of laboratory incidents via the submission of notification reports and follow-up report(s). A Customer Relationship Management system is used as the "inward facing" platform for capturing LINC data that is then exported to Excel for analysis.

For this report, data on laboratory incidents that occurred between January 1 and December 31, 2018 were extracted from the Customer Relationship Management system. Microsoft Excel 2016 was used for the descriptive analysis.

Exposure incidents included those with the potential to cause infection/intoxication and those leading to a confirmed or suspected laboratory-acquired infection involving RG2 to RG4 human pathogens and toxins that are within the scope of HPTA/HPTR. Excluded from the analysis were duplicate entries, and incidents and reports that were not within the scope of HPTA/HPTR, such as incidents involving RG1 human pathogens or pathogens in their natural environment.

Analysis

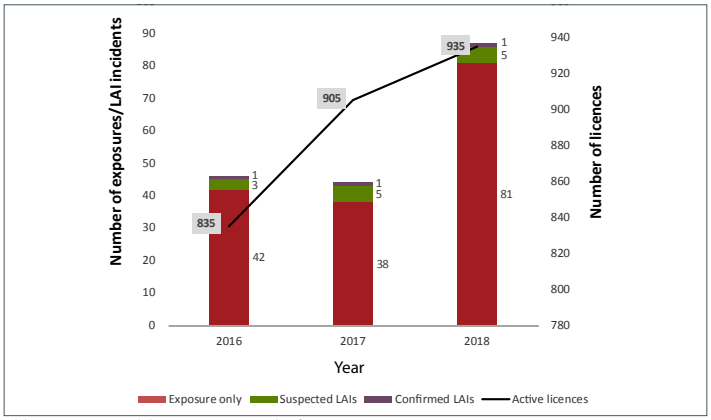
Data from reports submitted to the LINC were extracted into Microsoft Excel 2016. The total number of incidents per licence was first compared with reported data from 2017 and 2016. Laboratory incidents were then analyzed by sector, human pathogens or toxins involved, incident type, people exposed, route of exposure and root causes. Some trends were identified through qualitative analysis during compliance monitoring and verification activities.

Results

In 2018, there were 89 exposure incidents to human pathogens, 235 people were exposed and there was one confirmed laboratory-acquired infection. This was approximately twice the number of exposure incidents in 2018 (n=89) than in 2017 (n=44) and 2016 (n=46) (3,4). The number of confirmed laboratory-acquired infections remained the same (n=1) (**Figure 1).** As of December 31, 2018, there were 985 active licences; 461 (47%) were in the private industry sector, 203 (21%) in the hospital sector, 200 (20%) in academia, 94 (9%) in other (non–public health) government sector and 27 (3%) in the public health sector. Overall this represented about a 20% increase in the number of active licences, from 2016 (n=835). There was an

increase in the ratio of exposure incidents to active licences in 2018 (1:11) compared to 2017 (1:20) and 2016 (1:18) (data not shown).

Figure 1: Reported exposure, confirmed and suspected laboratory-acquired infection incidents and active licences, Canada, 2016–2018

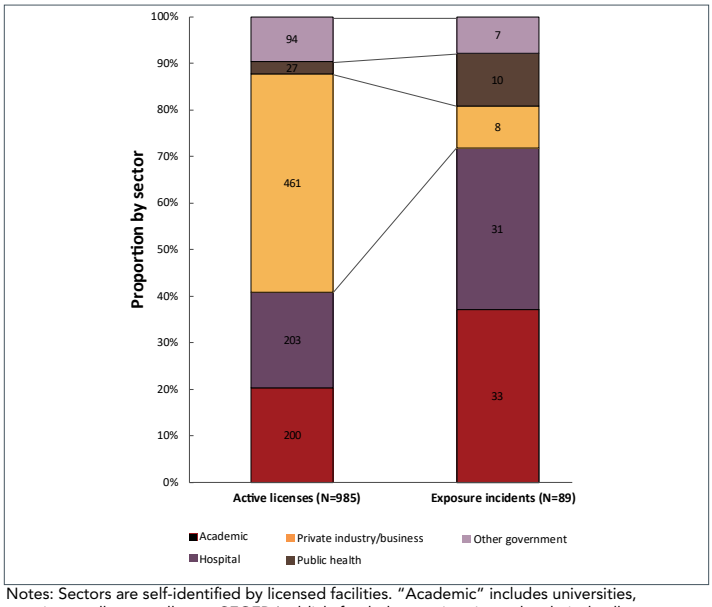


Abbreviation: LAI, laboratory-acquired infection
Sources: Data for 2018 were retrieved from the Laboratory Incident Notification Canada (LINC) system. Data for 2016 and 2017 were retrieved from published reports (5,6)

Exposure incidents by sector

In 2018, the highest number of exposure incidents occurred in the academic (n=33; 37%) and hospital sectors (n=31; 35%) (**Figure 2**). The private sector had the highest proportion of active licenses (n=461; 47%) and a fairly low proportion of exposures reported (n=8; 9%), thus leading to the lowest ratio of incidents to licence (1:58). The public health sector has the highest ratio of incidents to licences with 10 reported exposures and 27 active licences (1:3).

Figure 2: Active licences and exposure incidents by sector, Canada, 2018



Notes: Sectors are self-identified by licensed facilities. "Academic" includes universities, veterinary colleges, colleges, CEGEP (publicly funded pre-university and technical colleges in the province of Quebec) and others; "hospital" includes academic-affiliated and non-academic–affiliated hospitals; "private industry/business" includes animal health, human health, biotechnology, pharmaceutical and the food industry, and pathogen and toxin distributors; "public health" includes federal, provincial, territorial and municipal government laboratories; "other government" includes veterinary/animal health, environmental and other governmental laboratories at the federal, provincial, territorial and municipal level
Source: Laboratory Incident Notification Canada (LINC)

The majority of exposure incidents involving human pathogens and toxins classified as RG2 (n=50) occurred in the academic sector (n=27; 54%); followed by the hospital sector (n=8; 16%); and private sector (n=7; 14%). Exposure incidents with pathogens classified as RG3 (n=32) mostly occurred in the hospital sector (n=20; 63%); followed by the public health sector (n=5; 16%) and academic sector (n=4; 13%) (data not shown).

Human pathogens and toxins

Of the 89 exposure incidents, the majority (n=50; 56%) involved RG2 human pathogens, 32 (36%) involved RG3 pathogens and one (1%) involved a toxin (**Table 1**). A total of 18 (20%) incidents involved security-sensitive biological agents at the RG3 level. The most frequently involved biological agents were bacteria (n=46), viruses (n=17) and fungi (n=10).

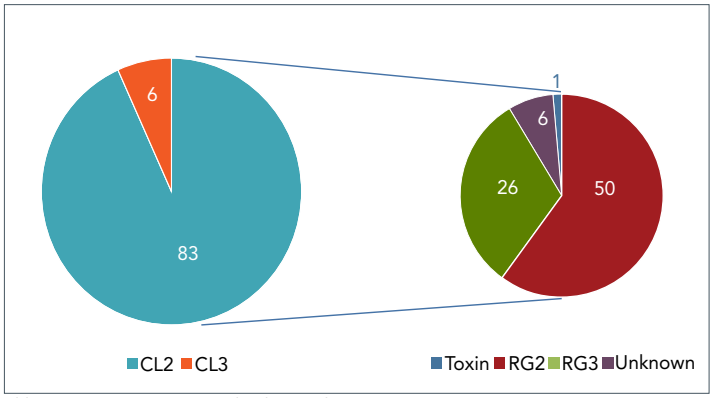
Table 1: Human pathogens and toxins involved in exposure incidents, by risk group level, Canada, 2018

Toxin or pathogen	Non SSBA		SSBA		Total	
	n	%	n	%	n	%
Toxin	1	1	–	–	1	1
RG2	50	77	–	–	50	56
Bacterium	30	46	–	–	30	34
Virus	12	18	–	–	12	13
Parasite	6	9	–	–	6	7
Prion	2	3	–	–	2	2
RG3	14	22	18	100	32	36
Bacterium	4	6	12	67	16	18
Virus	5	8	–	–	5	6
Fungus	4	6	6	33	10	11
Prion	1	2	–	–	1	1
Unknown	–	–	–	–	6	7
Total	65	100	18	100	89	100

Abbreviations: RG, risk group; SSBA, security-sensitive biological agent; –, non-applicable
[a] Toxins are not categorized by risk group. Toxins in the scope of the *Human Pathogens and Toxins Act* (HPTA) can be found in Schedule 1
Note: Percentages rounded to the nearest whole number
Source: Laboratory Incident Notification Canada (LINC)

Most exposure incidents occurred in Containment Level (CL) 2 laboratories (n=83; 93%) and the rest occurred in CL3 (n=6; 7%). Most incidents that occurred in CL2 laboratories involved a RG2 pathogen (n=50; 60%) (**Figure 3**).

Figure 3: Exposure incidents by containment level, and distribution of exposure incidents by risk groups in containment level 2 facilities, Canada, 2018



Abbreviations: CL, containment level; RG, risk group

There were 26 (29%) exposure incidents that involved an inadvertent possession of an RG3 biological agent in a CL2 laboratory (Figure 2). Of those, half (n=13; 50%) involved *Brucella melitensis* (n=7) and *Coccidioides immitis* (n=6) (**Table 2**), and mainly occurred in the hospital (n=11) sector. There was a spike in exposures to *Brucella* species, between March and November. Seven cases were reported, with the majority occurring in July and August. The confirmed laboratory-acquired infection involved *Salmonella enterica* subspecies *enterica* serovar Enteritidis.

Table 2: Numbers of laboratory incidents and exposed individuals by biological agent, Canada, 2018

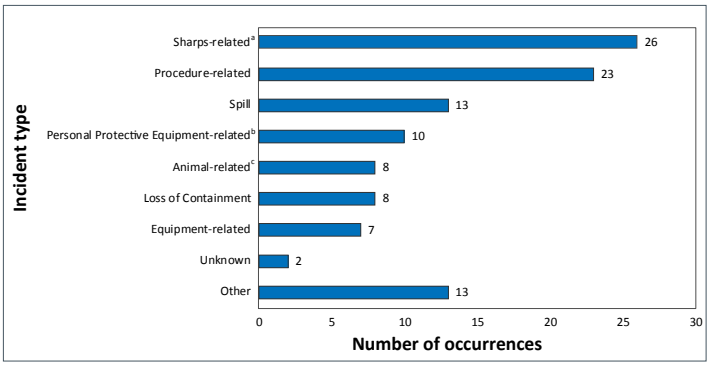
Biological agent	Incidents (N=89)	Exposed individuals (N=235)		
		Exposure only (n=229)	Suspected LAI (n=5)	Confirmed LAI (n=1)
RG2	50	63	4	1
Neisseria meningitidis	5	8	–	–
Staphylococcus aureus	3	3	–	–
Escherichia coli	2	4	–	–
Lymphocytic choriomeningitis mammarenavirus	2	2	1	–
Salmonella enterica	2	1	–	1
Other RG2 incidents	36	45	3	–
RG3	32	159	1	–
Brucella melitensis	7	105	1	–
Coccidioides immitis	6	15	–	–
Francisella tularensis	3	13	–	–
Mycobacterium tuberculosis	3	9	–	–
Other RG3 incidents	13	17	–	–
Toxins	1	1	–	–
Unknown	6	6	–	–

Abbreviations: LAI, laboratory-acquired infection; RG, risk group; –, non-applicable
Source: Laboratory Incident Notification Canada (LINC)

Incident types

There were 110 incident types identified for the 89 exposures reported. The most common types of exposure incidents were related to sharps (n=26; 24%) and procedures (n=23; 21%) (**Figure 4**). During a qualitative exposure incident review, a spike in incidents involving broken glass was identified. This was confirmed during on-site inspection.

Figure 4: Reported exposure incident type, Canada, 2018 (N=110)



Note: More than one incident type can be reported for the same incident
[a] Sharps-related includes needle sticks and other sharp injuries
[b] Personal protective equipment-related includes inadequate or failure of personal protective equipment
[c] Animal-related includes bites and scratches
Source: Laboratory Incident Notification Canada (LINC)

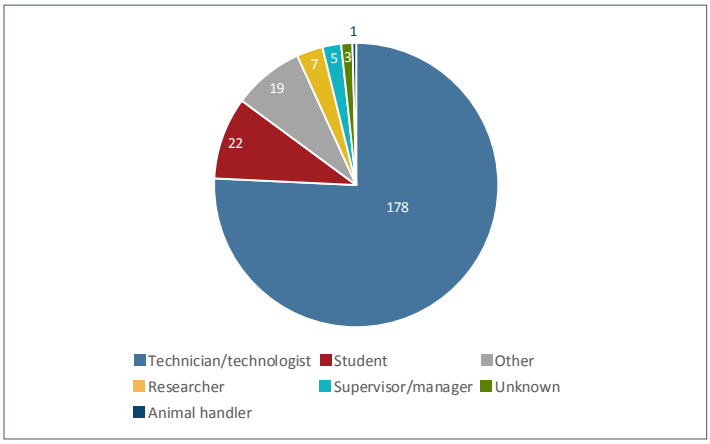
People exposed

In the 89 exposure incidents, 235 people were exposed to a human pathogen or toxin. Of these, five (2%) developed a suspected laboratory-acquired infection and one (0.4%) a confirmed laboratory-acquired infection. All six of the suspected or confirmed laboratory-acquired infections involved only one person exposed per incident.

In most exposure incidents (n=67; 75%), only one person was exposed. In 10 incidents (11%), 2–3 people were exposed and in seven incidents (8%), 4–10 people were exposed. There were missing data from two reports. The remaining incidents (n=3; 3%) involved 10 or more people exposed; these incidents occurred in the hospital sector, where 14, 29 and 53 people were exposed to *Brucella melitensis*.

Of the 235 people exposed, 39 (17%) received first aid treatment and 85 (36%) received prophylaxis within seven days of the incident. In addition, 8 (3%) people received postexposure prophylaxis more than seven days after the incident. The majority of people exposed were laboratory technicians (n=178; 76%) or students (n=22; 9%) (**Figure 5**).

Figure 5: Reported roles of people exposed, Canada, 2018 (N=235)

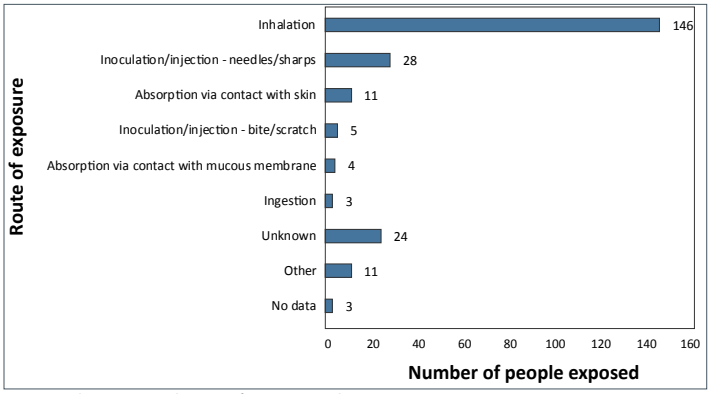


Source: Laboratory Incident Notification Canada (LINC)

Route of exposure

Of the 235 people exposed, the majority were exposed to pathogens or toxins through inhalation (n=146; 62%), while inoculation/injection of needles or sharps was the second most common route of exposure (n=28; 12%) (**Figure 6**).

Figure 6: Reported route of exposure, Canada, 2018 (N=235)



Source: Laboratory Incident Notification Canada (LINC)

Root causes and areas for improvement

In 2018, 233 root causes were identified for the 89 exposure incidents, an average of 2.6 root causes cited per incident. As shown in **Table 3**, human interactions and breaches to the standard operating procedures were the leading root causes (n=53; 23%, n=50; 22% respectively).

Table 3: Root causes and area of improvement reported for exposure incident involving human pathogens or toxins, Canada, 2018 (N=233)

Root cause/area of improvement	Example of areas of concern	Citations 2018	
		n	%
Human interactions	Workload constraints/ pressures/demands	53	23
Standard operating procedure	Procedures were not known /not followed correctly	52	22
Equipment	Equipment was not properly designed/maintained	32	14
Training	Training was not implemented or developed	27	12
Communication	There was no method or system for communication	24	10
Management and oversight	Supervision needed improvement	24	10
Other	Not applicable	21	9

Note: Percentages rounded to nearest whole number
Source: Laboratory Incident Notification Canada (LINC)

Discussion

Altogether, 235 people were exposed to human pathogens and toxins, through 89 exposure incidents. Of these, five incidents led to a suspected laboratory-acquired infection and one to a confirmed infection. Most exposure incidents occurred as a result of sharps and procedure breaches. Human interactions and lack of awareness or compliance with standard operating procedures were the leading root causes.

Although the overall annual reported incidence of laboratory exposures in Canada doubled in 2018 compared to the two previous years, it is not yet known if this rise represents a true increase in incidence or an increase in reporting because baseline estimates are still being established. The Centre for Biosecurity regularly conducts improvements to its LINC surveillance system to facilitate reporting and enhance clarity on regulatory requirements; it is possible that this has contributed to the increase in reporting. Since the LINC is still a fairly new surveillance system, it is likely that organizations will take some time to become accustomed to regulatory reporting requirements.

As in previous years, the number of incidents remained highest in the academic and hospital sectors (5,6). This is expected based on the difference between sectors in the nature of their work. Specifically, the identity of the biological agent is often unknown in hospital/diagnostic laboratories, increasing uncertainty of the risk status and the potential for exposure if the risk is underestimated. Private sector laboratories usually work

on samples where the pathogen is already known (e.g. for live vaccine development).

Technicians were involved in the highest number of laboratory exposures, but this might be because they represent the largest proportion of workers manipulating human pathogens and toxins. Technicians are present in all sectors, whereas animal handlers, for example, are not. Of laboratory workers, students had the most exposures incidents after technicians. Anecdotal evidence suggests that students in the academic sector may not be fully aware of or comply with laboratory procedures and safety measures, which may result in the high number of exposure incidents in this population.

Several key findings in this study concur with results reported in the literature: the majority of laboratory-acquired infections occurred in CL2 laboratories; spills and sharps-related occurrences were the most frequently reported types of incident; the main routes of exposure were inhalation and inoculation; and human error and problems with standard operating procedures were the most commonly reported root causes (7,8). The literature also shows that exposure to bacteria is more common than other pathogens (9,10). Specifically, *Brucella melitensis, Coccidioides immitis, Francisella tularensis* and *Mycobacterium tuberculosis* are among the most common biological agents involved in exposures and laboratory-acquired infections (11–13).

Strengths and limitations

The main strength of this study is that it is based on mandatory and standardized reporting and incorporates a review process that includes validation of the self-reported data. This aspect of the surveillance system allows timely and systematic reporting that enables the Centre for Biosecurity to assess the corrective measures that have been put in place by licensed facilities – to identify potential risk factors and to disseminate information. For example, as a result of detecting the spike in incidents involving *Brucella* species in 2018, an email blast was distributed advising facilities to increase biosafety vigilance (*Biosafety and Biosecurity for Pathogens and Toxins eBlast, Laboratory Incidents Involving Brucella species in 2018 – a spike in July, August 2018*). Similarly, stakeholders were notified of the potential risk of sharps-related exposures caused by broken glass and informed of techniques to mitigate the risk (*Biosafety and Biosecurity for Pathogens and Toxins Newsletter, Laboratory Incident Notification (LINC) Program Feature Report: Exposure Related to Broken Glass, October 2018*). Thus, this regulatory and surveillance program enables the early detection of common and emerging risks and the dissemination of information to increase awareness of both the risks and the best mitigation strategies to stakeholders across Canada.

The main limitation of this study is that data may be incomplete, as under certain circumstances laboratory incidents may not be detected or may simply not be reported due to lack of awareness of the requirements or a reluctance to report incidents. This continues to be addressed with the various publications, such as the notification and reporting guideline under the HPTA/HPTR, newsletters and biosafety advisories as well as compliance monitoring and verifications activities that aim to promote reporting and compliance. At this time, we neither have accurate data on the number of licensed facilities that are non-compliant to reporting requirements nor on the number of workers in laboratories. This makes it difficult to draw meaningful conclusions on the significance of ratios of reports by sector.

Next steps

In a number of areas, additional data and analysis would be relevant to the Centre for Biosecurity's activities. For example, to assess if students have an increased risk for exposures it would be useful to identify the number of workers in laboratories across Canada and to review the proportionality of students by both roles and sector. Such information would help the Centre for Biosecurity to better identify risk and allow for more targeted outreach and compliance promotion.

Conclusion

Although the annual incidence of laboratory exposures in Canada remains low, the incidence of laboratory exposures was higher in 2018 than in previous years. It is not yet known if this is a true increase in incidence or an increase in reporting as baseline estimates are still being established. Analysis of the reported exposures serve to inform and update biosafety standards and guidelines for ongoing improvement of biosafety in Canada.

Authors' statement

DC — Incident monitoring, data analysis – original draft, writing, review and editing
LS — Data analysis – original draft, writing, review and editing
SE — Writing – review and editing
FT and MH — Incidence monitoring, writing – review, editing and supervision
EF — Writing – review, editing and supervision

Conflict of interest

None.

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Appendix

Definitions relating to the *Human Pathogens and Toxins Act* (HPTA)

Term	Definitions
Biological safety officer (BSO)	An individual designated for overseeing the facility's biosafety and biosecurity practices.
Containment level (CL)	Minimum physical containment and operational practice requirements for handling human pathogens or toxins safely in laboratory environments. There are four containment levels, from a basic (CL1) to the highest (CL4) level.
Containment zone	A physical area that meets the requirements for a specified containment level. A containment zone can be a single room, a series of co-located rooms or several adjoining rooms. Dedicated support areas, including anterooms (with showers and "clean" and "dirty" change areas, where required), are considered to be part of the containment zone.
Exposure	Contact with, or close proximity to, human pathogens or toxins that may result in infection or intoxication, respectively. Routes of exposure include inhalation, ingestion, inoculation and absorption.
Exposure follow-up report	A tool used to report and document incident occurrence and investigation information for an exposure incident previously notified to the Public Health Agency of Canada.
Exposure notification report	A tool used to notify and document preliminary information to the Public Health Agency of Canada of an exposure incident.
Incident	An event or occurrence involving infectious material, infected animals or toxins that have the potential to result in injury, harm, infection, disease or cause damage.
Laboratory	An area within a facility or the facility itself where biological material is handled for scientific or medical purposes.
Laboratory-acquired infection/intoxication	Infection or intoxication resulting from exposure to infectious material, infected animals or toxins handled or stored in the containment zone.
Licence	An authorization to conduct one or more controlled activities with human pathogens or toxins issued by the Public Health Agency of Canada under Section 18 of the HPTA. One licence can cover many containment zones.
Risk group (RG)	The classification of biological material based on its inherent characteristics, including pathogenicity, virulence, risk of spread and availability of effective prophylactic or therapeutic treatments, that describes the risk to the health of individuals and the public as well as the health of animals and the animal population.
Security-sensitive biological agents (SSBAs)	The subset of human pathogens and toxins that have been determined to pose an increased biosecurity risk due to their potential for use as a biological weapon. Security-sensitive biological agents are identified as prescribed human pathogens and toxins by Section 10 of the *Human Pathogens and Toxins Regulations* (HPTR). This includes all Risk Group 3 and 4 human pathogens that are in the *List of Human and Animal Pathogens for Export Control*, published by the Australia Group, as amended from time to time, with the exception of Duvenhage virus, Rabies virus and all other members of the Lyssavirus genus, Vesicular stomatitis virus, and Lymphocytic choriomeningitis virus. This also includes all toxins listed in Schedule 1 of the HPTA that are listed on the *List of Human and Animal Pathogens for Export Control* when in a quantity greater than that specified in Section 10 (2) of the *Human Pathogens and Toxins Regulations*.
For more definitions, please see the Canadian Biosafety Standard, Second Edition (4)	

National Collaborating Centre for Infectious Diseases

Centre de collaboration nationale des maladies infectieuses

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