ORIGINAL ARTICLE

Comparison of clinical and epidemiological features of Shiga toxin-producing *Escherichia coli* O157 and non-O157 infections in British Columbia, 2009 to 2011

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INTRODUCTION: Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne agents that have the potential to cause severe enteric illnesses and large outbreaks worldwide. Several studies found non-O157 infections to be clinically milder than O157 STEC infections.

OBJECTIVE: To compare the clinical and epidemiological profiles of O157 and non-O157 STEC human infections in British Columbia (BC).

METHODS: All STEC cases reported in BC from 2009 to 2011 by four local health authorities were included in the study. Cases were classified according to STEC serotype based on laboratory information. Information was gathered via case interview forms. Data analysis included the χ^2 test and Mann-Whitney test; $P<0.05$ was considered to be statistically significant.

RESULTS: A total of 260 STEC cases were reported, including 154 (59.2%) O157 cases, 63 (24.2%) non-O157 cases and 43 (16.5%) STEC cases with no serotype identified. Hospitalization rate was higher and duration of hospitalization was significantly longer for O157 cases compared with non-O157 cases, but other clinical features were not significantly different. Patients with non-O157 infections were significantly more likely to have travelled outside Canada, less likely to report food exposure at social gatherings and more likely to consume bagged greens and cheese.

DISCUSSION: O157 is the predominant O serotype in BC and appeared to be more clinically severe than non-O157 STEC infections. However, the true incidence and severity of non-O157 remain unknown due to our current inability to detect all non-O157 cases. The present study and the literature suggest the need to identify more predictive virulence factors because serotype does not consistently predict disease severity.

Key Words: *Clinical severity; Enteric illness; Epidemiological profile; O157 serotype; STEC*

La comparaison des caractéristiques cliniques et épidémiologiques des infections à *Escherichia coli* producteur de Shigatoxine O157 et non O157 de 2009 à 2011 en Colombie-Britannique

INTRODUCTION : Les *Escherichia coli* producteurs de Shigatoxine (ECST) sont d'importants agents de toxi-infection alimentaire qui ont le potentiel de provoquer de graves maladies entériques et de vastes éclosions dans le monde. Selon plusieurs études, les infections à ECST non O157 sont plus modérées sur le plan clinique que celles à ECST O157.

OBJECTIF : Comparer les profils cliniques et épidémiologiques des infections humaines à ECST O157 et non O157 en Colombie-Britannique (CB).

MÉTHODOLOGIE : Les chercheurs ont inclus dans l'étude tous les cas d'ECST déclarés en CB entre 2009 et 2011 par quatre régies de la santé locales. Ils ont classé les cas selon le sérotype d'ECST tiré de données de laboratoire et ont obtenu de l'information au moyen de formulaires d'entrevue des cas. L'analyse des données incluait le test χ^2 et le test de Mann-Whitney, et le $P<0,05$ était considéré comme statistiquement significatif.

RÉSULTATS : Au total, 260 cas d'ECST ont été signalés, dont 154 cas O157 (59,2 %), 63 cas non O157 (24,2%) et 43 cas sans sérotype défini (16,5 %). Le taux d'hospitalisation était plus élevé et la durée d'hospitalisation considérablement plus longue dans les cas O157 que dans les cas non O157, mais d'autres caractéristiques cliniques n'étaient pas très différentes. Les patients atteints d'une infection non O157 étaient beaucoup plus susceptibles d'avoir voyagé à l'extérieur du Canada, moins susceptibles de déclarer avoir été exposés à des aliments lors de rencontres sociales et plus susceptibles d'avoir consommé des légumes verts emballés et du fromage

EXPOSÉ : Le sérotype O157 est le sérotype O prédominant en CB et semblait être plus grave sur le plan clinique que les infections à ECST non O157. Cependant, on ne connaît toujours pas la véritable incidence et la véritable gravité des infections non O157 en raison de notre incapacité à déceler tous les cas non O157. D'après la présente étude et les publications, il faudra déterminer des facteurs de virulence plus prédictifs, parce que le sérotype ne permet pas de prédire systématiquement la gravité de la maladie.

Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne agents that cause human enteric illnesses worldwide (1,2). The public health impact of STEC lies in their association with severe illnesses and their potential for causing large outbreaks. STEC infection may result in bloody diarrhea, hemolytic uremic syndrome (HUS) or even death (3). Between 2007 and 2011 in British Columbia (BC) (population 4.4 million), an average of 136 STEC infections were reported per year (4).

O157:H7 is the STEC serotype most frequently isolated in North America, although the incidence is decreasing (5-7), while infections caused by non-O157 STEC are more common in continental Europe (8-10). The burden of illness associated with non-O157 STEC in

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TABLE 1
Shiga toxin-producing *Escherichia coli* serotype distribution in British Columbia*, 2009 to 2011

Serotype	n	%
O157	154	59.2
O26	14	5.4
O121	8	3.1
O103	6	2.3
O111	6	2.3
O118	2	0.8
Other non-O157†	27	10.4
Unspecified	43	16.5
Total	260	100.0

**Includes data from four of five Health Authorities; †All Shiga toxin-producing* E coli *serotypes with <2 cases were grouped in this category*

North America is unknown due to limitations in laboratory testing protocols. In particular, the lack of verotoxin testing in many frontline laboratories results in an overall underestimation of the number of non-O157 STEC cases (11-13).

Previous studies have found non-O157 STEC infection to be clinically milder than O157 STEC infection in terms of the proportion of cases who had bloody diarrhea, were hospitalized or developed HUS (14-20). The risk factors also differed, with patients with non-O157 STEC being more likely to have travelled outside of North America and less likely to have eaten at a social gathering compared with O157 cases (15,17,21).

The present study compared the clinical and epidemiological profiles of O157 and non-O157 STEC human infections in the province of BC to assess whether they differ sufficiently to warrant a different public health response.

METHODS

In BC, all stool samples are routinely screened by frontline laboratories for O157 STEC using sorbitol MacConkey (SMAC) or similar agar. O157 STEC cannot ferment sorbitol and forms colourless colonies (SMAC-positive), while non-O157 STEC and other intestinal flora ferment sorbitol and form pink colonies. In most cases, cultures that are SMAC-positive are sent to the BC Public Health Microbiology & Reference Laboratory (PHMRL) for thorough organism identification and serotyping. Only bloody stools and stools from severe cases are forwarded directly to BC PHMRL without initial SMAC screening. At BC PHRML, the presence of Shiga toxin-producing organism in stool samples or cultures was confirmed using the Vero cell assay. If the assay result is positive, the Shiga toxin-producing organism is isolated and serotyped using the Kaufmann scheme (22-24).

STEC infection is a reportable disease in BC, even when no organism is identified in culture, and all patients are interviewed using a standard form (25). All STEC cases reported in BC from 2009 to 2011 from four of the five local health authorities (HA) (Fraser, Vancouver Island, Interior and Northern) were included in the present study (representing 76% of the population of BC). The number of interview forms was verified against reported cases in the integrated Public Health Information System, and the STEC serotype information was confirmed with the BC PHMRL reporting system or other laboratory reports. The data were entered in an electronic database (EpiData 3.1; EpiData Association, Denmark).

STEC cases were classified as O157 or non-O157, or were denoted as unspecified if a verotoxin-producing organism was identified by assay but *E coli* could not be identified in culture. Hospitalized cases were defined as patients staying at least 24 h in hospital, excluding emergency department visits. Travel-associated cases were defined as patients who travelled outside Canada during their incubation period. Travel-confirmed cases were defined as patients who travelled outside Canada for the entire duration of the incubation period (one to 10 days before the date of onset).

Demographic information, clinical features and exposure profiles of O157 and non-O157 STEC patients were compared and analyzed.

TABLE 2
Comparison of demographic features between O157 and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) serotypes in British Columbia*, 2009 to 2011

Variable	Total STEC (n=260)	O157 STEC (n=154)	Non-O157 STEC (n=63)	P
Age, years				
<10	56 (21.5)	45 (29.2)	6 (9.5)	0.002
≥10	204 (78.5)	109 (70.8)	57 (90.5)	
Sex				
Female	143 (55)	78 (50.6)	40 (63.5)	0.0847
Male	117 (45)	76 (49.4)	23 (36.5)	
Average annual incidence	2.6/100,000	1.5/100,000	0.6/100,000	

*Data presented as n (%) unless otherwise indicated. *Includes data from four of five Health Authorities*

Outbreak-associated cases were included for demographic and clinical comparisons, but only one randomly selected case from each outbreak was included in exposure comparisons; based on event location, time and laboratory information, the two outbreaks during the study period were both caused by a source common to all cases. STEC cases with unspecified serotypes were excluded from comparisons and confirmed travel cases were excluded from all other exposure analyses. Missing data were excluded from percentage calculation for clinical and exposure comparisons.

Population estimates were obtained from the BC Statistics website (26). Analysis was performed using Excel 2007 (Microsoft Corporation, USA), EpiDataStat, EpiCalc 2000 (version 1.02) (EpiData Association, Denmark) and the Wilcoxon-Mann-Whitney U Test Calculator (27). The χ^2 test was used to compare categorical variables and the Mann-Whitney test was used to compare median hospitalization duration. An alpha of 0.05 was used to determine statistical significance.

The present study was approved by the University of British Columbia (Vancouver, BC) Research Ethics Board.

RESULTS

A total of 260 STEC cases were reported between 2009 and 2011. There were 154 (59.2%) O157 cases, 63 (24.2%) non-O157 cases and 43 (16.5%) STEC-unspecified cases. The most common O serotype was O157 (Table 1). The age of all STEC patients ranged from one to 98 years, with a median age of 25 years.

Demographic comparisons (Table 2)

There was a higher proportion of children younger than 10 years of age among O157 patients compared with non-O157 patients (29.2% versus 9.5%; P=0.002). Non-O157 patients included a higher percentage of females than O157 patients, although this was not statistically significant. The average annual incidence over the study period was 1.5 per 100,000 for O157 STEC and 0.6 per 100,000 for non-O157 STEC.

Both O157 and non-O157 STEC showed summer peaks (Figure 1). During the study period, there were two O157 STEC outbreaks: eleven cases in September 2009 and five cases in April 2010.

Clinical comparisons (Table 3)

Of all O157 (n=154) and non-O157 (n=63) cases, hospitalization data were available for 137; of the 60 patients who were hospitalized, the duration of hospitalization was indicated for 38 patients. Based on available information, hospitalization rate was higher and the duration of hospitalization was longer for O157 patients compared with non-O157 patients (P=0.024 and P=0.044, respectively).

Nine (5.8%) of the O157 patients developed HUS while only one (1.6%) case of HUS was reported among non-O157 patients. However, the difference was not statistically significant (P=0.317). All patients with HUS were hospitalized.

A higher percentage of O157 patients reported each clinical symptom, including bloody diarrhea and fever, compared with non-O157

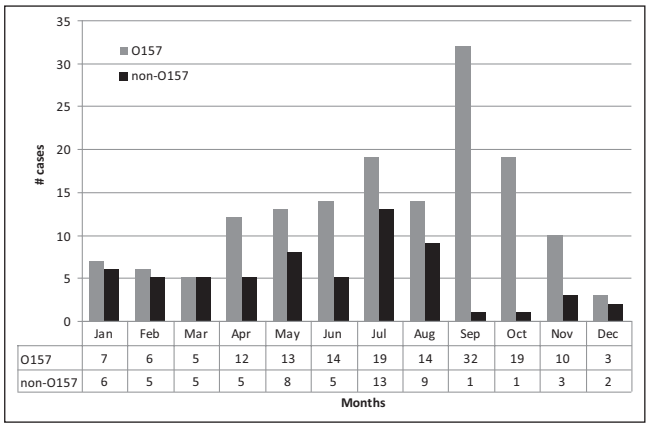

Figure 1) *Seasonal distribution of O157 and non-O157 Shiga toxin-producing* Escherichia coli *cases according to month in British Columbia, 2009 to 2011. Data from four of five Health Authorities are included. Includes eleven O157 cases in September (Sep) and five O157 cases in April (Apr) that were attributed to two outbreaks during 2009 to 2011. Aug August; Dec December; Feb February; Jan January; Jul July; Jun June; Mar March; Nov November; Oct October*

patients, but none of the differences were statistically significant. No deaths were reported at the time of case interviews.

Exposure comparisons (Table 3)

Non-O157 patients were more likely to have travelled outside Canada during their incubation period compared with O157 patients (P=0.001), and were more likely to acquire infection during travel, as shown by the difference in confirmed travel (P=0.037).

O157 patients reported a significantly higher percentage of having eaten at social gatherings (eg, parties, weddings, showers, potlucks, community events) compared with non-O157 patients (40.1% versus 12.1%; P=0.012). Non-O157 patients reported a significantly higher percentage of bagged greens and cheese consumption than O157 patients (P=0.014 and P=0.037, respectively). No other food (eg, ground beef, hamburgers, lettuce) or animal exposures (eg, farm, petting zoo, pets) were significant.

TABLE 3
Comparison of clinical information and selected high-risk exposures between O157 and non-O157 Shiga toxin-producing *Escherichia coli* cases, British Columbia*, 2009 to 2011

Variables	n	O157	Non-O157	P
Clinical				
Hospitalization	137	50 (49.5)	10 (27.8)	0.024
Duration of hospitalization, days, median (range)	38	4 (1–30)	2 (1–6)	0.044
Hemolytic uremic syndrome	217	9 (5.8)	1 (1.6)	0.317
Diarrhea	184	121 (93.8)	46 (83.6)	0.057
Vomiting	184	30 (23.3)	11 (20.0)	0.770
Nausea	184	35 (27.1)	12 (21.8)	0.057
Bloody diarrhea	184	92 (71.3)	36 (65.5)	0.538
Abdominal cramps	184	95 (73.6)	33 (58.2)	0.057
Fever	184	37 (28.7)	9 (16.4)	0.114
Exposure†				
Any international travel	181	19 (15.4)	26 (44.8)	0.001
Confirmed international travel	181	4 (3.3)	7 (12.1)	0.037
Social gathering	113	34 (40.1)	4 (12.1)	0.012
Bagged greens	108	24 (30.4)	17 (58.6)	0.014
Cheese	117	63 (74.1)	30 (93.8)	0.037

*Data presented as n (%) unless otherwise indicated. *Includes data from four of five Health Authorities; †Patients who answered "don't know" to an exposure question were excluded from analysis of that particular exposure*

DISCUSSION

The present initial STEC comparison study in BC showed that O157 is the predominant STEC serotype in the province and that O157 STEC infection differs from non-O157 STEC infection with regard to clinical severity indicators and exposure profiles. Non-O157 accounted for only 24% of all STEC cases in BC, which deviates significantly from recent estimates published by the CDC (64%) as well as the results obtained from enhanced surveillance in Manitoba (63%) (28,29). A ten-year analysis of STEC cases from Connecticut (USA), where Shiga toxin testing has been increasingly adopted to complement SMAC screening, also showed a higher percentage of non-O157 (58%) among all STEC cases (20). Therefore, the low proportion of non-O157 STEC in the present study is most likely due to the fact that most of the frontline laboratories in BC do not routinely test for non-O157 serotypes, leading to under-reporting. Geographical variation in the prevalence of STEC serotypes may also play a role. However, the most common serotypes in BC were O157, O26, O121, O103 and O111, which matches other study findings in both Canada and the United States (1,5,17,29).

O157 patients were more likely to be younger children than non-O157 patients in BC, while studies in other regions have shown conflicting findings with regard to age distribution. Compared with non-O157 patients, O157 patients were more likely to be children in a Connecticut study but more likely to be adults in New Mexico (USA). Therefore, age distribution may vary according to geographical location (17,20). There was no statistically significant difference in sex distribution between O157 and non-O157 cases, consistent with most comparison studies (15,17,21). Both O157 and non-O157 STEC occurred more frequently during the summer months with or without outbreak-associated cases, which is consistent with the literature evidence (5,15).

Overall, O157 patients were more severely ill. A higher proportion of O157 patients (49.5%) were hospitalized than non-O157 patients (27.8%), consistent with the literature (12,13,15,16,20). However, the proportion of hospitalization for non-O157 patients was higher than that in the United States (27.8% versus 12.8%) (28). Because most BC frontline laboratories do not routinely screen for non-O157, it is possible that only patients with more severe clinical presentation are tested for non-O157, leading to an artificially high proportion of hospitalization reported for non-O157 patients. O157 patients were significantly more likely to stay in hospital longer than non-O157 patients. However, when patients with HUS were excluded, statistical significance was not observed (data not shown). Therefore, the difference in duration of hospitalization is attributable to the long hospital stays of the nine O157 patients who developed HUS.

O157 STEC are most frequently associated with HUS worldwide; however, this does not apply to every country. For example, in Australia, HUS is more commonly associated with non-O157 STEC (30,31). In the present study, there was only one case of HUS reported among non-O157 cases. This was possibly due to the small sample size, the distribution of specific non-O157 serotypes or other virulence factors absent in BC, or that non-O157 is clinically milder than O157 STEC infections.

Consistent with studies from New Mexico, Connecticut and Minnesota, non-O157 patients were more likely to have travelled internationally than O157 patients (15,17,20). The percentage of O157 patients that ate at a social gathering was much higher, similar to findings in Argentina (21). O157 STEC may be more likely than non-O157 STEC to be associated with foods served at social gatherings, such as undercooked barbecued meat, but the comparison of specific foods did not support this hypothesis. The higher percentage of non-O157 patients who consumed bagged greens may be due to the higher percentage of females among non-O157 cases. Non-O157 STEC tend to grow more strongly and persistently in cheese than O157 STEC (32),

which could explain the higher percentage of non-O157 patients who consumed cheese. Overall, the similarity in food exposure among O157 and non-O157 patients may be because most of the foods, such as ground beef, hamburgers and lettuce, are commonly consumed by the general population in North America; therefore, any statistical difference can only be detected in a larger sample size.

Due to the current laboratory practices in BC, the available data on non-O157 STEC infections may be biased. Non-O157 STEC patients with less severe symptoms, such as nonbloody diarrhea, could easily go undetected, which could make non-O157 infection appear more severe clinically than it truly is. Improved detection of non-O157 serotypes at the front-line laboratories is needed to elucidate the exact burden of illness associated with non-O157 STEC infection in BC. Another limitation to the present study is the lack of data from one HA. This is, however, unlikely to significantly affect the study results because 76% of the BC population has been covered by the four HAs. The study also found a large number of verotoxin-positive organisms that could not be isolated, which limited the power of the study. The reason for this may be that the actual number of organisms was low and not uniformly distributed in the sample, or that the organisms were rendered unviable by the time the sample arrived in the laboratory. To increase sensitivity and maintain specificity, the BC PHMRL began using polymerase chain reaction for toxin detection in 2012. In addition, most interviews were conducted two to three weeks after symptom onset; some patients may not have developed HUS by that time and may not have left the hospital. Therefore, these variables may not contain complete data and the HUS incidence and hospital duration may be underestimated. Because of delay in reporting and the self-reported nature of the data, recall bias may exist.

The comparisons in the present study are based on O157 and non-O157 serotypes, but certain non-O157 serotypes, such as O26 and O111, can cause severe illness comparable to that caused by the O157 serotype (9,33). Because O157 and non-O157 serotypes do not appear to be consistently predictive of disease severity, it is crucial to identify other, more predictive virulence factors. Studies have suggested that STEC with the virulence gene *stx2* are associated with increased risk of HUS (5,34-36). Other virulence factors, such as the *eae* gene and cytotoxin SubAB, have also been suggested (9,33,34,37). More research is needed to advance our understanding of the pathogenesis of STEC.

SUMMARY

O157 is the predominant O serotype in BC. O157 STEC infections were more clinically severe than non-O157 STEC infections. However, due to our current limitations in detecting all non-O157 cases, the true incidence and severity remain unknown. In addition, more predictive virulence factors need to be identified to better inform public health practice.

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