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Prevalence and Risk Factors for the Contamination of Cattle Carcasses With Shiga Toxin-Producing *Escherichia coli* in Provincially Licensed Abattoirs in Ontario, Canada, Based on Molecular Surveillance

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ABSTRACT

Introduction: Reducing the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) is an important responsibility of provincial abattoirs to ensure safe products are entering the human food chain. Currently, within Ontario, provincial abattoirs are mandated to apply various antimicrobial treatments to cattle carcasses to help decrease pathogen presence post-slaughter. The objectives of this study were to determine the prevalence of contamination of O157 and non-O157 STEC in carcasses from Ontario provincial abattoirs.

Methods: Using mixed logistic regression models, we examined the associations between cattle characteristics, season, monitoring program and abattoir interventions on carcass contamination with *E. coli* O157:H7, non-O157:H7 STEC and the top six non-O157:H7 STEC of concern to public health (i.e., O26, O45, O103, O111, O121 and O145). Random effects for abattoir and the area in which an abattoir was located were included in these models to adjust for clustering at these levels. The STEC examined was detected through two provincial molecular-based monitoring programs.

Results: Samples taken in the summer had significantly greater odds of screening positive for the top six STEC compared to samples taken in the fall and winter months. Similar seasonal effects were observed for *E. coli* O157:H7, but for only one of the monitoring programs (i.e., seasonal effects were modified by a monitoring program). Carcasses that received dry age treatment had significantly lower odds of screening positive for STEC. Samples collected from veal calf and cow carcasses had significantly greater odds of screening positive for STEC than samples taken from the carcasses of steers or heifers, but not bulls. Most of the variance in carcass contamination was explained at the carcass level.

Conclusions: These results suggest that additional efforts in risk mitigation should focus on cattle of certain demographic characteristics and higher risk seasons and that additional carcass-level interventions be explored.

1 | Introduction

With 1 in 8 Canadians experiencing incidents of foodborne illness each year (Thomas and Murray 2014), food safety is an important responsibility of various government agencies and food industries. Shiga toxin-producing *Escherichia coli* (STEC) is one of the top 10 leading causes of foodborne disease in Canada (Thomas et al. 2013). STEC strains can be transmitted

All authors contributed equally to the work.

Summary

- *Escherichia coli* O157:H7 and other zoonotic STEC are being detected on cattle carcasses based on the current sampling program implemented at provincially licensed abattoirs in Ontario.
- Abattoirs should consider additional interventions in the warmer months and on cattle carcasses from certain animal classes (i.e., veal calves and cows) to further mitigate the presence of some STEC serotypes on carcasses.
- Dry age treatment appears to be the most effective intervention type to decrease the prevalence of STEC on carcasses. Further adoption of this intervention should be considered among Ontario abattoirs.
- A small proportion of the variance in the prevalence of carcass contamination with various serotypes of STEC is explained at the abattoir or regional level, suggesting that interventions should be focused at the carcass level.

through faecal contamination of surfaces, food and water (PHAC 2017a). Humans infected with certain STEC strains, including *E. coli* O157:H7, may experience the following signs and symptoms: bloody diarrhoea, severe stomach cramps, nausea, vomiting and complications associated with hemolytic uremic syndrome (CDC 2021). Therefore, mitigating the risk of exposure to harmful pathogens, such as STEC, is a continuously pressing issue for the food industry and governing bodies. One of the most common strains of STEC is *E. coli* O157:H7, and over 400 cases of infection with this serotype are recorded annually in Canada (PHAC, 2017b). Although the number of *E. coli* O157:H7 cases has remained steady for many years, non-O157:H7 STEC cases have continued to increase since 2010, with a recent report of an annual rate of 2.5 cases per 100,000 people (NESP 2020). The farm-to-fork concept has been implemented by many countries to help decrease the risk of exposure to foodborne pathogens by increasing interventions along the farm-to-fork continuum.

Cattle can be asymptomatic carriers of STEC and have been identified as an important reservoir in contaminating the environment as well as food derived from plants and animals (Gonzalez and Cerqueira 2019). STEC can be present on cattle hides and within faecal matter and gut contents (Stromberg et al. 2018). In a recent study conducted in Michigan, the prevalence of STEC within faeces was about 8% higher in beef cattle compared to dairy cattle (Venegas-Vargas et al. 2016). Maintaining clean hides and withdrawing feed for a short time prior to transport to an abattoir can help mitigate the risk of carcass contamination at slaughter (Ontario 2022). Although on-farm techniques to control and minimise *E. coli* O157:H7 will not eradicate the bacteria, they can decrease the likelihood that carcasses will be contaminated during processing (Soon et al. 2011). Using both pre- and post-harvest interventions to reduce the risk of contamination of dairy and beef products has proven to be the most effective approach (Soon et al. 2011). Many foodborne illnesses have been linked to the consumption or mishandling of beef products intended for consumption (McEvoy et al. 2003; Essendoubi et al. 2019). Controlling the rate of contamination that occurs when handling and processing meat is an important step to ensure the public are consuming safe products (Rhoades et al. 2009). The prevalence of *E. coli* O157 and non-O157 STEC from faecal samples from cattle at slaughter can range from 0.2% to 27.8% and 2.1% to 70.1%, respectively (Hussein and Bollinger 2005). Consequently, abattoir hygienic practices can play an important role in mitigating contamination of carcasses with STEC (Essendoubi et al. 2019).

Within Canada, processing of cattle for human consumption is regulated by provincial or federal inspectors to ensure a safe product. Provincially licensed abattoirs are prohibited from distributing products outside of their province and are often operating on a smaller scale than federally licensed plants. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) employs provincial inspectors to help implement requirements from the *Food Safety and Quality Act, 2001* to ensure a safe product is being approved for consumption by the public (Ontario 2022). Various antimicrobial interventions are used in abattoirs across Ontario, Canada including but not limited to, organic acid rinses, hot water rinses, dry aging and carcass washing (Ontario 2022). Antimicrobial procedures involving hot water and/or steam are effective at reducing bacterial pathogens when the surface of a carcass reaches temperatures greater than 70°C (Antic et al. 2021). Bacterial populations can also be effectively reduced with chemical washes, especially those containing lactic, peracetic, chlorine or citric acids (Antic et al. 2021; Government of Canada 2022). Trimming and washing a carcass can be quite effective, but outcomes are greatly dependent on an operator's abilities (Antic et al. 2021). As per *O. Reg. 31/05* s. 104 (1) (b), OMAFRA requires all meat products that are produced to not be contaminated, and in July 2019, regular monitoring of the presence of O157 and non-O157 STEC on cattle carcasses was made mandatory across the province (Ontario 2022).

The objectives of this study were to determine the following: prevalence of contamination of carcasses with O157 and non-O157 STEC using molecular screening; identify the association and potential influence of cattle characteristics, season, treatment, number of treatments and sampling program on the prevalence of O157 and non-O157 STEC contamination and determine how much of the variance in carcass contamination is explained by abattoir effects. This study will help provide insight on the relative performance of current measures used within provincially licensed abattoirs to reduce STEC contamination of carcasses, identify carcasses at higher risk of contamination and assist in planning improved intervention and surveillance strategies to reduce the incidence rate of O157 and non-O157 STEC cases in Ontario.

2 | Materials and Methods

2.1 | Ethics

Ethics approval was not required for this study, as it did not involve live human or animal subjects. The research focused on the examination of prevalence of STEC on cattle carcasses at provincially licensed abattoirs in Ontario, Canada. As such, there were no interactions with living beings or invasive procedures

conducted. All data were obtained from routine sampling procedures conducted by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and no identifiable information was used in analysis.

2.2 | Sample Collection

During the study period, the OMAFRA Meat Inspection Program required all provincially licensed abattoirs that slaughter beef to undergo regular monitoring for the presence of *E. coli* O157:H7 and the top six STEC (i.e., O26, O45, O103, O111, O121 and O145) on carcasses. Each sample collected was tested, and results were recorded and stored in the OMAFRA Enterprise Platform, a Siebel Systems Inc. application (Oracle, Austin, TX) and Labvantage (Chatterjee Group, Somerset, NJ). The dataset for this study contained the testing results from 2246 samples collected from all 85 abattoirs that process cattle across the province from July 2019 to May 2022.

Risk-based information paired with volume of slaughter was considered in creating the sampling plan for each individual abattoir. All abattoirs that processed beef during the study period were included. Provincial abattoirs are much smaller with cattle slaughtered per week ranging from 10 to 500, compared to federal abattoirs that process ~60,000 cattle a week (Government of Canada 2021). In practice, rather than design, inspectors used convenience sampling based on their scheduled activities rather than probability sampling to select carcasses throughout each month. Samples were collected by swabbing a 100 cm^2 area of the posterior left and right sides of the carcass, and the anterior left and right sides of the carcass using separate moistened sponges for each area. Samples were collected from each carcass after evisceration, final trimming and the application of the microbiological intervention method(s) used by the abattoir. Samples were taken prior to chilling unless dry aging was used as an intervention method. Samples were labelled and packed into coolers with ice packs and shipped to the Agriculture and Food Laboratory in Guelph, Ontario.

2.3 | Sample Analysis

The anterior left and posterior left swabs of each carcass sampled were combined at the lab to test for the STEC genes. These samples were screened for the presence of STEC genes using the BAX (Hygiena, Camarilla, CA) System with real-time polymerase chain reaction (PCR). A sample was classified as being positive for STEC when a Shiga toxin gene (i.e., stx_1 and/or stx_2) and the intimin gene (i.e., *eae*) were detected within the sample at concentrations as low as 10^4 cfu/mL after enrichment. A sample was STEC-negative if Shiga toxin genes or the intimin gene were not detected. Once a sample was deemed screen-positive for STEC, further testing via an additional two panel assay was used with the same lysate to determine if the sample was also positive for one of the top six STEC genes (i.e., O26, O45, O103, O111, O121 and O145) using the BAX (Hygiena, Camarilla, CA) System with real-time PCR.

To test for the presence of *E. coli* O157:H7, the anterior right and posterior right swabs of each carcass sampled were combined at the lab and real-time PCR was performed with the BAX System; this screening test can detect *E. coli* O157:H7 at concentrations as low as 10^4 cfu/mL after enrichment.

2.4 | Data

The data were collected by the Meat Inspection Program within OMAFRA. Prior to January 2021, the sampling program (Project 5020) was a random sampling program where a computer randomly generated the number of carcass samples required to be taken at each abattoir monthly. In January 2021, the program switched to become a constant monitoring program (Project 1120) where abattoirs were tested monthly, with frequency being determined based on the number of cattle slaughtered per year and other in-plant risk factors. One carcass sample per month was required from abattoirs that slaughtered up to 1000 cattle in a year, and two carcass samples per month were required from abattoirs that slaughtered more than 1000 cattle in a year. Other factors affecting the frequency of sample collection included high frequency of positive swab results and results from a daily hygienic slaughter checklist, but these data were not available for subsequent analyses. Data concerning plant and carcass characteristics were provided to the researchers by OMAFRA in an Excel (Microsoft Office, Redmond, WA) spreadsheet which was imported into STATA 17 Statistical Software (StataCorp, College Station, TX).

The plant-level variables collected included area where the plant was located (for administrative purposes, OMAFRA split the province into 10 areas, each with a similar number of abattoirs), the abattoir identification number and the type and number of interventions used by the abattoir. Each abattoir was given a random and unique identifier code to maintain anonymity. Interventions were classified into three groups: acid treatment, hot water treatment and dry age treatment. Acid treatment was defined as the application of any form of acid intervention pre-chilling including acetic acid, peracetic acid, sodium hypochlorite, citric acid and lactic acid. Hot water treatment was defined as the application of hot water to the carcass pre-chilling at a temperature greater than 74°C. Dry age treatment was defined as chilling a carcass for a minimum of 6 days in a cooler that is less than 4°C with a consistent relative humidity level under 90% recorded daily by the abattoir. Other variables available included the date the sample was taken on, the product class of the animal sampled and the sampling program at the time of sample collection. Sample collection dates were classified into seasons: fall (September–November), winter (December–February), spring (March–May) and summer (June–August). In the original dataset, product class included bulls, steers, heifers, cows, heavy veal calves, dairy veal calves, veal calves male and veal calves female. To ensure sufficient numbers for subsequent statistical analyses, all subcategories of veal calves were combined into one category.

2.5 | Statistical Analysis

Descriptive statistics for each dependent and independent variable were reported which included frequencies and proportions. For dependent variables (i.e., O157, STEC and top

six STEC status), the raw prevalence and 95% confidence intervals were reported. The correlation between independent variables was examined using Phi correlation coefficients. To avoid potential issues with collinearity, two variables were not included in the same multivariable model if their correlation was >0.7. In addition, treatment type and number of treatments were not included in the same model since they were related through construction.

Mixed effects univariable logistic regression models were fitted to identify associations between the three outcomes (i.e., O157, STEC and top six STEC status) and the following independent variables: season, product class, type of treatment, number of treatments and project code. Random intercepts were included in univariable and multivariable models to account for clustering by abattoir and the areas in the province where abattoirs were located. A random intercept was only removed from the final multivariable model if the variance component was less than 1×10^{-3} and its removal did not impact the direction or statistical significance of model coefficients.

Prior to fitting multivariable models, a causal diagram was created to identify relationships between the independent variables and VTEC outcomes being explored (i.e., O157, STEC and Top six STEC status). The diagram helped identify extraneous variables, such as confounding and intervening variables. Model coefficients and odds ratios were interpreted using the causal diagram, along with comparison between univariable and multivariable analyses, to determine whether full or direct effects were measured or if variables were excluded from the multivariable model due to the inclusion of an intervening variable. A manual backward elimination process was used while fitting the multivariable models for each dependent variable. If an independent variable was statistically significant, part of a statistically significant interaction, or acted as an explanatory antecedent or distorter variable (i.e., a confounding variable), it was included in the final model. A confounding variable was defined as a non-intervening variable that, if removed from the model, caused a 20% or greater change in the coefficient of a statistically significant variable. Interactions between treatments, season and type of treatment, season and number of treatments, season and project code, season and product class and project code with every other variable were tested. A variable or interaction term was considered statistically significant based on a significance level of 5% ($\alpha=0.05$). Wald chi-square tests were used to assess the statistical significance of variables and interaction terms with more than two categories. Contrasts were used to examine differences among statistically significant variables with more than two categories and different covariate patterns when examining statistically significant interactions. Individual contrasts were examined only if the global test for the variable's or interaction term's coefficients was statistically significant. No further adjustments for multiple comparisons were performed after assessing the overall significance of the coefficients. The assumptions of normality and homoscedasticity of the best linear unbiased predictions (BLUPs) were examined visually by examining normal quantile plots and scatter plots of the BLUPs against the predicted outcome, respectively. Outliers among observations were identified by examining Pearson residuals. Variance partition coefficients were estimated using the latent variable technique to determine what proportion of the total variance is attributable to the carcass, abattoirs and area level.

3 | Results

3.1 | Descriptive Statistics

Based on genetic characteristics, the prevalence of O157, STEC and any of the top six STEC was 4.9%, 6.4% and 3.5%, respectively (Table 1). Acid treatment was the most common intervention agent used among sampled carcasses (92.7%), and most carcasses were only treated with one intervention (91.2%) to reduce bacterial contamination (Table 2). In terms of cattle characteristics, there were more samples taken from steers (33.9%) than any other bovine class (Table 2). Most samples were obtained from project 1120 (61.9%), and the greatest number of samples were obtained in the spring (27.4%) (Table 2).

3.2 | Mixed Logistic Regression Analyses

3.2.1 | *E. coli* O157:H7

Based on univariable analyses, treatment, cattle characteristics, and season had no statistically significant associations with *E. coli* O157 status of tested carcasses (Table 3).

In the multivariable analysis, we identified a statistically significant interaction between season and project code (Table 4). The odds of a project 1120 sample screening positive for O157 were significantly lower when taken in the fall than in the spring and summer (Table 5). The odds of a project 1120 sample

TABLE 1 | Carcass level prevalence of genes related to the identification of *E. coli* O157:H7, Shiga toxin producing *E. coli* (STEC), and the Top six[a] STEC of concern among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Number of carcasses sampled	Prevalence of gene(s) (%)	95% confidence intervals of prevalence estimate (%)
O157 status	2246	4.9	4.0, 6.0
STEC status	2246	6.4	5.0, 7.0
Top six STEC status	2246[b]	3.4	3.0, 4.0

[a]O26, O45, O103, O111, O121 and O145.
[b]Samples were only tested for Top 6 STEC genes if they screened positive for STEC in the first panel assay.

TABLE 2 | Descriptive statistics concerning the treatments used, number of treatments used, classification of bovine, project code, and season each sample was taken among tested cattle carcasses from Ontario provincial abattoirs (2019–2022).

Variable	Number of carcasses (abattoirs)[a]	Percentage of carcasses
Acid treatment		
Acid not used as intervention	165 (6)	7.4
Acid applied as intervention	2081 (81)	92.7
Hot water treatment		
Hot water not used as intervention	2055 (65)	91.5
Hot water applied as intervention	191 (22)	8.5
Dry age treatment		
Dry age not used as intervention	2093 (71)	93.2
Dry age used as intervention	153 (16)	6.8
Number of treatments		
One treatment	2049 (58)	91.2
More than one treatment	197 (29)	8.8
Product class		
Bulls	263	11.7
Cows	140	6.2
Heifers	727	32.4
Steers	761	33.9
Veal calves	355	15.8
Project code		
1120[b]	1390	61.9
5020[b]	856	38.1
Season		
Fall	589	26.2
Winter	550	24.5
Spring	615	27.4
Summer	492	21.9

[a]Number of abattoirs using a particular treatment.
[b]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

TABLE 3 | Results of mixed[a] univariable logistic regression concerning the association between O157 status and season, product class, type of treatment, project code, and number of antimicrobial treatments performed among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Odds ratio	95% confidence interval	*p*
Season			0.100[b]
Fall	referent		
Spring	1.30	0.77, 2.20	0.316
Summer	1.46	0.85, 2.50	0.166
Winter	0.73	0.39, 1.35	0.316
Product class			0.574[b]
Bulls	referent		
Cows	0.45	0.15, 1.39	0.167
Heifers	0.74	0.40, 1.37	0.332
Steers	0.77	0.41, 1.43	0.408
Veal calves	0.97	0.50, 1.90	0.931
Acid treatment			
No	referent		
Yes	2.15	0.78, 5.94	0.140
Hot water treatment			
No	referent		
Yes	0.61	0.26, 1.42	0.255
Dry age treatment			
No	referent		
Yes	0.51	0.18, 1.40	0.190
Project code			
5020[c]	referent		
1120[c]	0.88	0.59, 1.30	0.507
Number of treatments			
One treatment	referent		
More than one treatment	0.59	0.25, 1.37	0.218

[a]Random intercepts included for abattoir.
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

screening positive for O157 were significantly higher when the sample was taken in the summer than the winter (Table 5). The odds of a project 5020 sample screening positive for O157 were significantly higher than a project 1120 sample in the fall (Table 5). The abattoir and carcass levels accounted for 2.1% and 97.9% of the variance, respectively.

TABLE 4 | Results of mixed[a] multivariable logistic regression concerning associations between O157 status and season, project code, and their interaction in tested cattle carcasses from Ontario provincial abattoirs (2019–2022).

Variable	Odds ratio	95% confidence interval	*p*
Season			0.359[b]
Fall	referent		
Spring	0.58	0.21, 1.58	0.285
Summer	0.85	0.42, 1.72	0.657
Winter	0.44	0.16, 1.20	0.108
Project code			
5020[c]	referent		
1120[c]	0.26	0.09, 0.70	0.008
Season project code[b]			0.04[b]
Spring 1120[b]	6.06	1.51, 24.2	0.011
Summer 1120[b]	4.69	1.37, 16.1	0.014
Winter 1120[b]	4.22	0.99, 17.9	0.051

[a]Random intercept included for abattoir (Var=0.07; 95% CI [0.002, 2.90]).
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

3.2.2 | STEC

Based on univariable analyses, the following variables had statistically significant associations with STEC status: season, product class, dry age treatment and project code (Table 6).

The final mixed effects multivariable model included these statistically significant variables: product class, dry age treatment and project code. The odds of a sample screening positive for STEC were significantly lower when dry aging was used as a treatment to the carcass prior to sampling (Table 7). The odds of a sample screening positive for STEC were significantly lower when the sample was taken as a project 1120 sample compared to a sample for project 5020 (Table 7). The odds of a sample screening positive for STEC were significantly higher when the sample was taken from a veal calf compared to steers or heifers (Table 8). When a sample was taken from steers or heifers, the odds of screening positive for STEC were significantly lower when compared to cows (Table 8). The abattoir area, the abattoir and the carcass level accounted for 2.1%, 1.2% and 96.7% of the variance, respectively.

3.2.3 | Top Six STEC—O26, O45, O103, O111, O121 and O145

Based on univariable analyses, season and project code had a statistically significant association with top six STEC status (Table 9). The dry age variable could not be explored for this outcome since none of the abattoirs using this treatment had a positive carcass for those serotypes.

The final mixed effects multivariable model included the following statistically significant variables: season and project code (Table 10). The odds of a sample screening positive for any of the top six STECs were significantly lower when the sample was taken during project 1120 compared to project 5020 (Table 10). When a sample was taken in the summer, the odds of testing positive for any of the top six STECs were significantly higher when compared to fall, spring and winter

TABLE 5 | Contrasts[a] examining the interactions between season and project code on the odds of a carcass sampled from Ontario provincial abattoirs screening positive for *E. coli* O157:H7.

Contrast	OR	95% CI	*p*
Project 5020 & Fall vs Project 5020 & Spring	1.73	0.63, 4.70	0.285
Project 5020 & Fall vs Project 5020 & Summer	1.17	0.58, 2.36	0.657
Project 5020 & Fall vs Project 5020 & Winter	2.27	0.84, 6.14	0.108
Project 5020 & Spring vs Project 5020 & Summer	0.68	0.24, 1.93	0.468
Project 5020 & Spring vs Project 5020 & Winter	1.31	0.37, 4.65	0.674
Project 5020 & Summer vs Project 5020 & Winter	1.93	0.68, 5.49	0.215
Project 1120 & Fall vs Project 1120 & Spring	**0.29**	**0.11, 0.74**	**0.010**
Project 1120 & Fall vs Project 1120 & Summer	**0.25**	**0.09, 0.69**	**0.007**
Project 1120 & Fall vs Project 1120 & Winter	0.53	0.19, 1.52	0.243
Project 1120 & Spring vs Project 1120 & Summer	0.88	0.47, 1.63	0.676
Project 1120 & Spring vs Project 1120 & Winter	1.88	0.97, 3.67	0.063
Project 1120 & Summer vs Project 1120 & Winter	**2.15**	**1.02, 4.52**	**0.040**
Project 5020 & Fall vs Project 1120 & Fall	**3.83**	**1.42, 10.34**	**0.008**
Project 5020 & Spring vs Project 1120 & Spring	0.63	0.24, 1.67	0.356
Project 5020 & Summer vs Project 1120 & Summer	0.82	0.39, 1.70	0.588
Project 5020 & Winter vs Project 1120 & Winter	0.91	0.32, 2.59	0.856

Note: All bolded values are statistically significant OR values where the *p* value is <0.05.
[a]From O157 mixed multivariable logistic regression model (see Table 4).

TABLE 6 | Results of mixed effects[a] univariable logistic regression concerning the associations between STEC status and season, product class, type of treatment, project code, and number of antimicrobial treatments performed among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Odds ratio	95% confidence interval	*p*
Season			0.0087[b]
Fall	referent		
Spring	0.52	0.31, 0.88	0.014
Summer	1.27	0.81, 1.99	0.293
Winter	0.88	0.55, 1.41	0.595
Product class			0.0007[b]
Bulls	referent		
Cows	2.04	1.01, 4.12	0.046
Heifers	0.68	0.38, 1.24	0.209
Steers	0.78	0.43, 1.41	0.409
Veal calves	3.45	1.21, 9.90	0.021
Acid treatment			
No	referent		
Yes	1.81	0.76, 4.34	0.182
Hot water treatment	referent		
No			
Yes	0.86	0.42, 1.73	0.664
Dry age treatment			
No	referent		
Yes	0.29	0.09, 0.93	0.037
Project code			
5020[c]	referent		
1120[c]	0.53	0.37, 0.75	0.0003
Number of treatments			
One treatment	referent		
More than one treatment	0.44	0.19, 1.01	0.05

[a]Random intercepts included for abattoir and area where an abattoir was located.
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

(Table 11). The abattoir area and the abattoir each accounted for 6.8% of the variance, and the carcass level accounted for 86.4% of the variance.

TABLE 7 | Results of mixed effects[a] multivariable logistic regression concerning associations between STEC status and product class, dry age treatment, and project code performed among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Odds ratio	95% confidence interval	*p*
Product class			0.006[b]
Bulls	referent		
Cows	1.97	0.97, 3.99	0.061
Heifers	0.70	0.39, 1.28	0.252
Steers	0.83	0.46, 1.49	0.527
Veal calves	1.42	0.75, 2.67	0.271
Dry age treatment			
No	referent		
Yes	0.28	0.09, 0.92	0.035
Project code			
5020[c]	referent		
1120[c]	0.52	0.36, 0.73	< 0.001

[a]Random intercepts included for each abattoir (Var=0.04; 95% CI [0.002, 0.92]) and area where abattoirs were located (Var=0.07; 95% CI [0.004, 1.2]).
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

3.3 | Diagnostics

The BLUPs for the O157 and top six STEC models met the assumption of homoscedasticity but were slightly skewed right when assessed for normality. The BLUPs for the STEC model appeared heteroscedastic and were slightly right-tailed when assessed for normality. No outliers were identified for all three models.

4 | Discussion

OMAFRA has assisted provincially licensed abattoirs to implement multiple strategies to help address the concerning issue of STEC contamination on cattle carcasses including mandating the application of antimicrobial interventions such as acid, hot water and/or dry age treatments. These treatments, along with external factors such as season and animal class, can all impact the prevalence of O157 and non-O157 STEC on cattle carcasses. The major goals of this study were to analyse current measures implemented at provincial abattoirs in Ontario, Canada, and assess where improvements could be applied in the post-harvest stage of the farm-to-fork process and identify types of carcasses and periods when risk of contamination is greatest. Samples collected over the course of 3 years from provincial abattoirs provided insight into factors

TABLE 8 | Contrasts[a] examining the effects of each product class on the odds of a carcass sampled from Ontario provincial abattoirs screening positive for STEC.

Contrast	OR	95% CI	*p*
Cows vs. Bulls	1.97	0.97, 3.99	0.061
Heifers vs. Bulls	0.70	0.39, 1.28	0.252
Steers vs. Bulls	0.83	0.46, 1.49	0.527
Veal calves vs. Bulls	1.42	0.75, 2.67	0.271
Veal calves vs. Steers	**1.72**	**1.01, 2.92**	**0.045**
Veal calves vs. Heifers	**2.02**	**1.18, 3.45**	**0.010**
Veal calves vs. Cows	0.72	0.38, 1.39	0.329
Steers vs. Heifers	1.17	0.73, 1.89	0.513
Steers vs. Cows	**0.42**	**0.23, 0.78**	**0.006**
Heifers vs. Cows	**0.36**	**0.19, 0.67**	**0.001**

Note: All bolded values are statistically significant OR values where the *p* value is <0.05.
[a]From STEC mixed multivariable logistic regression model (Table 7).

TABLE 9 | Results of mixed[a] univariable logistic regression concerning the association between Top six STEC status and season, product class, type of treatment, project code, and number of antimicrobial treatments performed among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Odds ratio	95% confidence interval	*p*
Season			0.0004[b]
Fall	referent		
Spring	0.96	0.47, 1.98	0.917
Summer	2.74	1.46, 5.14	0.002
Winter	1.01	0.48, 2.09	0.988
Product class			0.250[b]
Bulls	referent		
Cows	1.73	0.67, 4.49	0.260
Heifers	0.84	0.38, 1.83	0.660
Steers	0.79	0.35, 1.76	0.560
Veal calves	1.42	0.61, 3.32	0.420
Acid treatment			
No	referent		
Yes	5.73	0.76, 42.96	0.090
Hot water treatment			
No	referent		
Yes	0.47	0.14, 1.58	0.220
Project code			
5020[c]	referent		
1120[c]	0.52	0.32, 0.83	0.006
Number of treatments			
One treatment	referent		
More than one treatment	0.30	0.07, 1.30	0.108

[a]Random intercepts included for each abattoir and area where abattoirs were located.
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

that increase the prevalence of O157 and non-O157 STEC on cattle carcasses. These samples were collected during the COVID-19 pandemic, which affected other commodity groups in the industry. However, the plants involved in this study maintained normal processing capacity. Based on molecular testing, the prevalence of carcasses from STEC remains a public health concern. Of the samples screening positive for STEC, 55% screened positive for the top six STEC, consistent with the increasing public health concern about non-O157:H7 STEC cases within Canada (NESP 2020).

Season was significantly associated with the prevalence of O157 and the top six STEC, but not with overall STEC contamination. Previous studies have found that cattle shed *E. coli* O157 at a much higher rate in warmer months as compared to colder months (Barkocy-Gallagher et al. 2003; Vidovic and Korber 2006), consistent with these findings. Additionally, most of the abattoirs are located within Southern Ontario, which experiences very hot and humid summer months, where O157 would likely thrive since the ideal temperature for survival ranges from 4.4°C to 49°C, with an optimal temperature for growth being 37°C (WHO 2018). In our study, samples taken in the summer months had higher odds of screening positive than samples taken in other seasons for the top six STEC with similar measures of association in univariable and multivariable models, but season interacted with project code in our O157 model. However, where significant differences were found between seasons, prevalence was higher in warmer months. Notably, seasonal differences within Project 5020 (the initial surveillance project) were not observed for the O157 model. It remains unclear why the seasonal effect varied by sampling program or secular time for one outcome (O157) but not the other (top six STEC), apart from potential issues related to statistical power. A significant association between season and overall STEC contamination may not have been identified in our study due to the less specific nature of the outcome compared to models focused on a single or small group of zoonotic serotypes of STEC. However, other variables in the multivariable model (e.g., product class and dry age treatment) may have acted as intervening variables, as season was significantly associated with STEC contamination in the univariable analysis.

Multiple hurdle interventions are commonly implemented at federal plants both in Canada and the United States (Stanford 2022; Koohmaraie et al. 2005). Application of multiple interventions

TABLE 10 | Results of mixed[a] multivariable logistic regression concerning associations between Top six STEC status and season and project code among tested cattle carcasses from Ontario provincial abattoirs (2019-2022).

Variable	Odds ratio	95% confidence interval	*p*
Season			0.001[b]
Fall	referent		
Spring	1.17	0.56, 2.48	0.680
Summer	2.83	1.50, 5.32	0.001
Winter	1.14	0.54, 2.40	0.730
Project code			
5020[c]	referent		
1120[c]	0.57	0.35, 0.93	0.023

[a]Random intercepts included for each abattoir (Var=0.24; 95% CI [0.03, 1.96]) and area where abattoirs were located (Var=0.24; 95% CI [0.03, 1.70]).
[b]Overall *p*-value calculated using the Wald Chi Square test.
[c]Project 5020 ran from July 1st, 2019-December 31st, 2020; Project 1120 began January 1, 2021, and is the current monitoring project.

TABLE 11 | Contrasts[a] examining the effect of each season on the odds of a carcass sampled from Ontario provincial abattoirs screening positive for any of the Top six STEC.

Contrast	OR	95% CI	*p*
Spring vs. Fall	1.17	0.56, 2.48	0.680
Summer vs. Fall	**2.83**	**1.50, 5.32**	**0.001**
Winter vs. Fall	1.14	0.54, 2.40	0.730
Summer vs. Winter	**2.48**	**1.31, 4.70**	**0.005**
Winter vs. Spring	0.97	0.47, 2.01	0.945
Summer vs. Spring	**2.42**	**1.28, 4.58**	**0.007**

Note: All bolded values are statistically significant OR values where the *p* value is <0.05.
[a]From Top six STEC mixed multivariable logistic regression model (Table 10).

throughout the slaughter process have proven to greatly decrease the prevalence of pathogens on carcasses at final examination (Antic et al. 2021). The number of treatments did not have a statistically significant effect on the prevalence of O157, STEC and the top six STEC in our study. This could mean that multiple interventions used consecutively might not be more beneficial in eliminating pathogens on carcasses as compared to one treatment or that certain plants utilising this should re-focus attention on other critical control points (CCPs) during dressing to improve hygienic slaughter as interventions cannot scavenge high loads of pathogens. Samples taken from carcasses that had been treated with dry age treatment had lower odds of screening positive for STEC than samples taken from carcasses that were not treated with this method. In addition, none of the carcasses that received dry age treatment tested positive for any of the top six STEC, but this prevented proper estimation of this treatment effect using mixed models for these serotypes. Although we did not find that dry aging impacted prevalence of *E. coli* O157 contamination, a study found that dry aging reduces the presence of O157:H7 on cattle carcasses significantly after 7 days and even more so at 21 and 28 days post intervention (Tittor et al. 2011). Although this research was on the effect of dry aging on the prevalence of O157:H7 on cattle carcasses, we found a similar effect on non-O157 STEC. The effect of dry aging on non-O157 STEC remained consistent across our univariable and multivariable models, with little change in the measure of association even after adjusting for potential confounders including product class and project code. Carcasses that undergo dry age treatment must remain in a cooler for a minimum of 6 days at a temperature less than 4°C. As *E. coli* survives at temperatures greater than 4.4°C, this prolonged period beneath the survival threshold should decrease the prevalence of these bacteria including STEC serotypes. With only 18% of abattoirs (Table 2) currently using this treatment type, it would be beneficial to examine the impact of greater adoption of this practice. Current research findings indicate that lactic acid and hot water use are the most effective at mitigating pathogen presence on carcasses during slaughter (Thomas et al. 2021; Wheeler et al. 2014), but there were no statistically significant associations found with any of the three outcomes examined. The high adoption of these practices among abattoirs coupled with the low prevalence of those outcomes may have limited our ability to observe their effects. Regarding interventions, our analysis was limited to examining the potential interactions described. Some interventions may interact with other plant processes or characteristics that were not available or recorded in the data available to our research team. However, no statistically significant interactions were identified among the interventions investigated.

The odds that a sample would screen positive for STEC were higher when taken from veal calves as compared to steers or heifers. Some research has found that younger animals are more likely to shed STEC, which is consistent with a higher prevalence of contamination for veal carcasses (Stein and Katz 2017; Thomas et al. 2021). It has also been found that age is an important factor associated with STEC shedding in cows, with 2-year old cows shedding the most when compared to younger heifers or older cows (Stein and Katz 2017). We found that samples taken from cows were more likely to screen positive for STEC than those taken from heifers and steers, but the odds of contamination were not greater for carcasses from veal calves and bulls. The odds ratios for product class were similar in our univariable and multivariable analyses. Operators of abattoirs should consider implementing increased hygienic practices on veal calves and cows to help decrease STEC presence at final carcass washing. There were no statistically significant associations between product class and O157 or the top six STEC. The power to detect an effect with breed class would be lower for these rarer outcomes, but differences in host dynamics with different serotypes of STEC need to also be investigated in the future.

The original design of these monitoring programs intended for carcasses to be selected randomly for testing. However, in practice, selection was more haphazard and influenced by the inspectors' scheduled activities. In addition, some inspectors were observed on occasion to preferentially select poorly dressed carcasses. This preferential selection could have increased the observed prevalence and potentially introduced selection bias.

Additionally, microbiological tests were not performed on samples to confirm PCR results; therefore, the load of viable *E. coli* O157, STEC and the top six STEC was not measured. It would be beneficial to perform quantitative microbiological testing to address the performance of interventions more accurately since these may decrease the concentration of these bacteria but not the prevalence. Additionally, improvements to plant CCPs were being made continuously through time when the initial project code, 5020, was introduced. The more recent monitoring program, 1120, had more abattoirs satisfying the requirements for proper CCPs and regular intervention use, leading to a difference in results observed between the two project codes. The differences in results associated with project code could also reflect the annual variation of *E. coli* O157 and STEC within the environment. Year and monitoring program could not be analysed within the same model due to complete correlation (i.e., the monitoring programs changed with year). Consequently, we elected to model the monitoring program, acknowledging the inability to fully differentiate between the effects of time and program.

Random effects for abattoir and abattoir area appeared to have modest effects on the variance in all three models, indicating that most of the variance is attributed to the carcass level. The impact of farm or clustering by a lot of cattle arriving at the abattoir could not be assessed since these data were not available or collected. However, it may be beneficial to include these variables in future studies to further examine the variance that is attributable to the farm/lot or events that transpire on a specific day at an abattoir. Understanding how much of the variance is explained at these additional levels will better clarify where to target interventions. Provincial abattoirs currently only implement one treatment type at minimum to mitigate the prevalence of STEC on cattle carcasses. Federally inspected plants have multiple interventions implemented at various points throughout the slaughter process. It would be beneficial to see how implementing interventions sooner in the slaughter process in our provincial abattoirs would help decrease the prevalence of these pathogens on carcasses entering the human food chain.

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Conflicts of Interest

Sarah Adam worked as a Food Safety Inspector with OMAFRA from March 2020 to May 2022.

Data Availability Statement

The data that support the findings of this study are available on request from the Ministry of Agriculture, Food and Rural Affairs. The data are not publicly available due to privacy or ethical restrictions.

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