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Research article

Seasonal effect on *Salmonella,* Shiga toxin-producing *E. coli* O157:H7 and non-O157 in the beef industry in Colombia, South America

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ABSTRACT

This research investigated the variations in the occurrence of *Salmonella*, STEC O157:H7 and non-O157 in the beef production chain in Colombia affected by seasons, hypothesizing that pathogen prevalence will be highest in the rainy season owing to soil moisture promoting bacteria multiplication and transfer between animals. To test this hypothesis, samples were obtained from five abattoirs, which represent 50% of the beef production in this country. A total of 1017 samples were collected, from which 606 were bovine feces, 206 were hide swabs, and 205 corresponded to carcass post-intervention. From the 1017 samples, 49.9% (n = 507) were collected during dry season, while 50.1% (n = 510) during rainy season. All samples (n = 1017) underwent screening for *E. coli* O157:H7 and *Salmonella*, while only a proportion of fecal samples (n = 339) were screened for the big six STEC serogroups and their virulence markers. The effect of season, age of animal and sex of animal were correlated with the prevalence results. A total of 84.7% of fecal samples carried virulence genes associated to STEC (*stx* or *eae*), suggesting that testing and control should be increased for the big-six STEC compared to *E. coli* O157:H7. Pathogen prevalence in feces was found to be 8.3%, 5.0%, and 51.0% for *Salmonella*, *E. coli* O157:H7 and STEC non-O157, respectively. Hides had a prevalence of 15.0% and 6.8% of *Salmonella* and *E. coli* O157:H7, respectively. Carcasses post-intervention were found to have 4.4% and 2.5% prevalence of *Salmonella* and *E. coli* O157:H7, respectively. A seasonal effect was found for fecal samples. *E. coli* O157 and non-O157 STEC shedding were significantly higher ($P \leq 0.05$) during rainy season compared to dry season. In contrast, hides and carcasses were more likely to present lower incidence of pathogens during rainy months compared to dry season; however, it was significant only for *Salmonella* on carcasses with estimated odds of detection almost six times higher in the dry season relative to the rainy season (OR = 5.90, 95% CI 1.18–29.57).

1. Introduction

Shiga toxin-producing *E. coli* (STEC) O157 and non-O157 along with *Salmonella* remain as the most important food safety concerns associated with beef consumption. Cattle are common carriers of these microorganisms in their feces, transferring them to hides and carcasses during slaughtering operations (Elder et al., 2000). According to the Centers for Disease Control and Prevention (CDC), the number of illnesses related to STEC and *Salmonella* in the United States are approximately 265,000 and 1.2 million per year, respectively in the US (CDC, 2018). Furthermore, as of 2016 the CDC reported an upward trend in illnesses related to various foodborne pathogens including *Salmonella* and STEC (CDC, 2018; Marder et al., 2017).

STEC and *Salmonella* represent a food safety burden worldwide. The Food and Agriculture Organization (FAO) under the umbrella of the World Health Organization (WHO) conducted a data analysis of STEC illnesses and deaths from around the world and identified that in 2010, about 2.5 million cases of STEC occurred worldwide with 269 deaths (WHO, 2018). On the other hand, Majowicz et al. (2010) investigated the global burden of gastroenteritis caused by *Salmonella*. The group

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synthesized data published in multiple surveillance studies from around the globe. Their findings indicate that around the globe, approximately 94 million people acquire Salmonellosis every year and about 155,000 die from the illness.

Statistics pertaining illness and outbreaks is scarce in Latin America. Nonetheless, considering the available reports, it is fair to say that in Latin American countries, enterohemorragic *E. coli* is an important agent causative of enteric infections. STEC illnesses and Hemolytic Uremic Syndrome (HUS) have been reported in South America since the early 60's in Argentina, being this country the one with the highest HUS incidence in the world (Chamorro, 2009). Other countries such as Colombia, Peru, Brazil, Uruguay, Paraguay, and Chile have sufficient data indicating the threat that these food-borne pathogens pose (Guth et al., 2010). Food-borne outbreak information is rare and difficult to find for South American countries. As indicated by Galli et al. (2016), most of these countries don't conduct case-control studies to investigate outbreaks and are unable to define the magnitude and spread of such illnesses.

Given the significance of these bacterial pathogens to the public health, the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) instituted a sampling program including *Salmonella* as well as STEC O157 and non-O157 to verify that slaughtering plants are properly implementing HACCP and ensuring pathogen process control (USDA, 2018a). Data collected from these types of sampling program provides baseline information that serves both, industry and government to understand the current situation as it pertains to those pathogens, establish goals aimed to reduce public health risks, and implement measures to achieve the food safety goals.

Pathogen prevalence studies in beef cattle are more common in the US, and infrequent in other countries and such is the case of Latin America. A very comprehensive study was conducted in the US by Barkocy-Gallagher et al., in 2003, investigating the effect of the seasons and type of samples on pathogen prevalence. Their findings revealed some variations between *Salmonella*, STEC O157 and non-O157 based on seasons (summer, fall, winter, spring) and sample type (hide, feces, carcasses). During summer months, a prevalence of 12.9% of *E. coli* O157:H7 was found in feces, while *Salmonella* and non-O157 STEC was 9.1% and 22.5%, respectively. These microorganisms were usually found more frequently in hides at 73.5, 91.6, and 56.1% for *E. coli* O157, *Salmonella*, and non-O157 STEC, respectively. However, there was a lower prevalence in carcasses than hides and feces. Their study also revealed that seasonal changes influence pathogen prevalence with the lowest levels during colder or winter months.

Unfortunately, pathogen baseline studies and prevalence data related to beef production are extremely scarce in Latin America. In some cases, countries lacking this critical information, back some regulatory and food safety inspection decisions based on the US regulations and public health information available from trading partners countries. Lower income countries usually don't have a comprehensive food safety surveillance program that provide consistent information to address food safety issues. In some cases, when samples are collected, the results obtained by health and inspection government agencies are not necessarily made available to the public. To date, there is no scientific research conducted to estimate the prevalence of STEC O157, non-O157, and *Salmonella* in the beef production chain in Colombia. This research seeks to provide objective information to the public that can be used to make science-based decisions pertaining microbial testing, food safety inspections, surveillance, and implementation of control measures to reduce microbial hazards in slaughtering houses.

2. Materials and methods

2.1. Sample collection

This project consisted on a nationwide prevalence study conducted in Colombia, South America. To select participating abattoirs, a data research was conducted to identify the biggest plants in Colombia that operate under official veterinarian inspection following HACCP requirements. Also, the number of beef cattle head in the country and the volume slaughtered per day in each plant was identified. Samples were obtained from five large abattoirs that receive cattle produced in various regions (Figure 1) and slaughter approximately 50% of the bovine cattle consumed in the country. These five abattoirs kill approximately 1000, 800, 400, 350, and 120 animals per day, respectively. A total of 1017 samples were collected over a period of three years between 2017 and 2019. Samples were collected during rainy season (50.1% of samples, n = 510) and dry season (49.9% of samples, n = 507) in a total of four collection time points (two visits per season). Samples corresponded to fecal grabs (n = 606), hides (n = 206), and carcass post-evisceration (n = 205). Fecal samples were obtained directly from the rectum of the shackled carcass during the slaughtering process at the bunging station (prior bunging), before evisceration. To collect each fecal grab, sterile palpation gloves were used per sample and discarded upon sample collection. Feces were aseptically placed into sterile sampling cups and labeled accordingly. Hide samples were collected using pre-moistened sponges with 25-ml buffered peptone water (World Bioproducts, Mundelein, IL, USA) by swabbing an area of ca. 8000 cm^2 from the inside round and the navel-plate- brisket-foreshank areas. Hides were swabbed when the animal was shackled, after stunning and before bleeding. On the other hand, carcass samples were collected during chilling about 12 h post-intervention; collection method, supplies, surface area, and location (on the carcass) was consistent with the method applied to hides. Each sample was labeled and detail information per sample was obtained such as collection date, city where the abattoir is located, and name of the plant. Animal information was also recorded including tag or identification number, age, sex, and growing location. All samples were transported in coolers with ice packs to maintain refrigeration conditions and shipped same day to the food microbiology laboratory at Pontificia Universidad Javeriana in Bogota, Colombia, for further analysis.

2.2. Sample preparation and enrichment

A portion of 10g of fecal samples were enriched into 90 ml of modified Tryptic Soy Broth (mTSB, Neogen Corporation, Lasing, MI, USA) containing casaminoacids and 8 mg/l of novobiocine. Samples

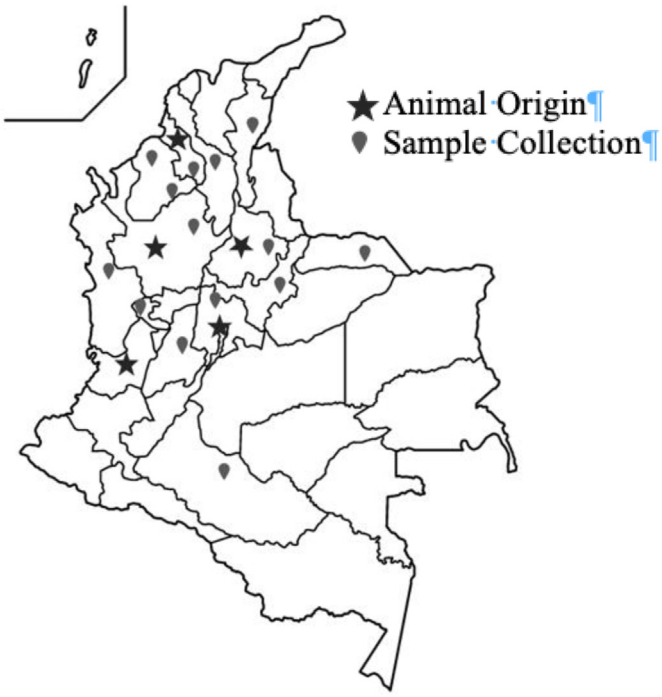

Figure 1. Sample collection and animal production areas.

were homogenized using a stomacher at 230 rpm for 2 min, and incubated for 18 h at 42 °C. Due to the high background microbiota present in feces, a 10-fold dilution was performed after the incubation time by placing 1 ml of the overnight fecal enrichment into 9 ml of mTSB, followed by a subsequent incubation at 42 °C for 12 h, in agitation. This last step facilitated also to reduce solids from the bovine feces, which could potentially interfere with the further molecular detection technique applied. Hide and carcass swabs were homogenized as described for the fecal samples. A portion of 10 ml from the sponge swab was transferred into 90 ml mTSB, and incubated for 24 h at 42 °C.

2.3. *E. coli* O157:H7, *Salmonella*, and STEC screening

Screening for E. coli O157:H7 and *Salmonella* was conducted to all samples (feces, hides, and carcass) using real-time and standard PCR BAX® System Q7 (Hygiena, Wilmington, DE, USA). The manufacturer's instructions were followed for detection. The big six non-O157 Shiga toxin-producing *E. coli* (STEC), as recognized by USDA were detected only in fecal samples (n = 339). The big six STEC included *E. coli* serotypes O111, O121, O103, O45, O145, and O26. STEC screening was also conducted using the BAX® System Q7 real-time PCR assay, which consisted of three separate testing steps; the first allowed for the screening of virulence marker genes *stx* and *eae*; if both genes are present, samples are considered as potential positive for STEC. Consequently, two separate real-time PCR screening kits were used. The first one known as "panel one" screens for STEC O111, O121, and O26, while the second one or "panel two" screens for STEC O103, O145, and O45.

In brief, a portion of the enriched sample –5 μl for Salmonella and 20 μl for *E. coli* O157 and non-O157 STEC—was added into a cluster tube containing 200 μl of the BAX® lysis reagent. For the lysis, cluster tubes were subjected to warming at 37 °C for 20, followed by heating at 95 °C for 10 min, and subsequent cooling at 4 °C for 5 min. To perform the real time PCR assay *E. coli* O157:H7, STEC Screening (*stx* and *eae*), STEC Panel 1, and STEC Panel 2, 30 μl of the lysate were added to hydrate the PCR tablets. To perform standard PCR assay for *Salmonella* detection, PCR tablets were hydrated with 50μl of the lysate. PCR tubes were covered with optical caps and loaded into the BAX® System Q7. The detection of all microorganisms did not undergo any confirmatory step or colony isolation.

2.4. Statistical analysis

To identify if the occurrence of these pathogens in Colombia was affected by climate, prevalence was calculated for each of the three pathogens (*E. coli* O157, non-O157, and *Salmonella*) and each type of sample (feces, hide, and carcass) by season (rainy and dry). Data was pooled for all the five locations in the country. For each prevalence estimate, exact binomial 95% CI were calculated using the SAS software (Brown et al., 2001). The software was also used to test for differences in sample-type prevalence estimates between seasons for each pathogen ($P < 0.05$). Logistic regression models were estimated to identify factors associated with the prevalence of *E. coli* O157, *Salmonella* spp. and non-O157 (outcome variables) for each sampling site. Three factors were included as explanatory variables into the models: season (rainy and dry), sex (steers and cows), and age of the animals. Moreover, to account for the hierarchical nature of the data and clustering, type of outlet was included as a random variable (Li et al., 2011). Likelihood ratio tests were used to assess the need to estimate models with random intercepts instead of ordinary logistic regression models. A Pearson χ^2 test was used to assess the validity of the models. After model estimation, odds ratios (OR) and confidence intervals 95% (CI) were calculated. All statistical procedures were performed using SAS. A value of $P < 0.05$ was considered as significant.

3. Results

The five participating meat processing plants are responsible for slaughtering about 50% of the cattle produced in Colombia, and those animals originated from different regions around the country. Data collected from the different plants was intended to represent the country; therefore, overall results are presented and will not be differentiated by abattoir. Fecal, hide, and carcass samples were randomly collected at the participating abattoirs and represented different breeds, sex classes, and age. There was no attempt to match specimens from the same animal when collecting fecal, hides, and carcass samples. All samples (n = 1017) underwent screening for *E. coli* O157:H7 and *Salmonella*, while only a proportion of fecal samples (n = 339) were screened for the big six STEC serogroups and their virulent markers. All samples in which the presence of the target pathogens was reported, are considered "potential positives" since culturing or colony isolation was not conducted. This consideration was made based on the criteria used by USDA FSIS Microbiology Laboratory Guidebook (MLG) 5.09 (2015) and MLG 5C.00 (2019).

3.1. *Salmonella* screening

The overall *Salmonella* prevalence was 8.8% (90 of 1017). However, when results were categorized per sample type it was found that hides had the highest prevalence at 15.0% (31 of 206), followed by feces with 8.3% (50 of 606), while carcasses had the lowest *Salmonella* prevalence with 4.4% (9 of 205). When results were broken down by season, dry or rainy (Table 1), no significant differences ($P > 0.05$) were found between the three types of samples.

3.2. *E. coli* O157:H7

An overall *E. coli* O157:H7 prevalence of 4.8% (49 of 1017) was found in the samples. Similar to *Salmonella*, hides had the highest *E. coli* O157:H7 prevalence at 6.8% (n = 30), followed by feces at 5.0% (14 of

Table 1. Seasonal variation in the prevalence of *E. coli* O157, *Salmonella*, and non-O157 STEC.

Season	*Salmonella*			*E. coli* O157:H7			Non-O157 STEC		
	n	% positive	95% CI	n	% positive	95% CI	n	% positive	95% CI
Feces									
Dry	300	6.33	3.86–9.71	300	3.00 A	1.38–5.62	146	50.68 A	42.29–59.05
Rainy	306	10.13	6.99–14.07	306	6.86 B	4.30–10.30	193	67.88 B	60.79–74.40
Hides									
Dry	104	15.38	9.06–23.78	104	7.69	3.38–14.60			
Rainy	102	14.71	8.47–23.09	102	5.88	2.19–12.36			
Carcasses									
Dry	103	6.80	2.78–13.50	103	2.91	0.60–8.28			
Rainy	102	1.96	0.24–6.90	102	1.96	0.24–6.90			

Within a sample type, values with different letters that are in the same column are statistically different (α = 0.05).

Table 2. Frequency of STEC serogroups in fecal samples.

O157 and non-O157	O111	O121	O26	O103	O145	O45	Other non-O157*
14	7	71	36	55	30	99	43

* Samples carrying *eae* and *stx* but not identified by PCR as one of the big six.

206), and carcasses with 2.4% (5 of 205). When samples were compared by season (Table 1), a significant difference ($P \leq 0.05$) was observed only in fecal samples with highest levels during rainy season. Hides and carcasses did not appear to be significantly different ($P > 0.05$) between rainy and dry season.

3.3. Non-O157 STEC screening and virulence markers

Results revealed that 51.0% (173 of 339) were potentially non-O157 STEC in fecal samples (as per USDA FSIS definition in MLG 5C.00). This in other words, corresponds to the proportion of samples carrying both virulent genes *stx* and *eae* simultaneously. From the STEC potential positive samples, 10.4% (18 of 173) did not belong to any of the "big six" non-O157 STEC. Furthermore, 28.6% (97 of 339) of the total samples carried only stx gene, while 2.4% (8 of 339) carried only eae gene. This means that 82.0% of the fecal samples tested (278 of 339) carried at least one or the combination of both, *eae* or *stx* genes identified as virulence markers for STEC. Statistical analyses indicate that during rainy season, the STEC prevalence in feces was significantly ($P \leq 0.05$) higher compared to the dry season as shown in Table 1. Out of the 173 non-O157 STEC potential positive samples, 8.1% (14 of 173) were also potential positive for *E. coli* O157:H7. It was common to find that samples carried more than one STEC O-group. From the STEC positive samples, 34.7% (60 of 173) carried only one STEC serogroup, 27.7% (48 out 173) carried two, 9.8% (17 out 173) carried 3, 6.9% (12 of 173) carried 4, 2.3% (4 out 173) carried 5, and 1.2% (2 of 173) carried the six STEC serogroups. With respect to the frequency of each serogroup in the fecal samples, PCR results indicate that the most recurrent serogroup was O45, followed by O121, O103, O26, O145 and the less frequent being O111 (33.2, 23.8, 18.5, 12.1, 10.1, and 2.3%, respectively). Table 2 depicts the frequency of each serogroup as they were distributed in the positive samples.

3.4. Seasonal, age and sex effect in prevalence

Consistent with the prevalence statistics shown in Table 1, regression results indicate that in feces, *E. coli* O157, non-O157, and *Salmonella* were less likely to be found during the dry than in the rainy season as the odds ratios were less than 1, even after controlling for age and sex of the animals. However, season was only significant in the case of non-O157 (OR = 0.47, 95% CI 0.30–0.74) and closely to be significant for *E. coli* O157:H7 (OR = 0.45, 95% CI 0.20–1.00) (Table 3). Regression results also indicate that in fecal samples, *E coli* O157 was more likely to be detected from cows than in steers (OR = 2.03, 95% CI 0.91–4.55). On the other hand, *Salmonella* and non-O157 STEC were less likely to be detected in cows (OR = 0.71, 95% CI 0.33–1.53; 0.64, 95% CI 0.37–1.12, respectively), although the sex of the animals was not statistically significant in any of the pathogens.

In the case of hides and carcasses, *E coli* O157:H7 and *Salmonella* were found more likely to be detected in the dry season than in the rainy season. This effect was significant only in the case of *Salmonella* on carcasses where the estimated odds of detection were almost six times higher in the dry season relative to the rainy season (OR = 5.90, 95% CI 1.18–29.57). In hides, both *Salmonella* and *E. coli* were more likely to be detected in cows than in steers whereas the opposite was found for carcasses, but the effect was only significant in the case of *Salmonella* in hides (OR = 3.24, 95% CI 1.20–8.74).

Finally, with regard to the age of the animal, an increase in the age of the animal was consistently found to be associated with a decrease in the likelihood of detecting *E. coli* O157:H7 and *Salmonella* in all sample types,

Table 3. Odds Ratios (OR) with 95% Confidence intervals (CI) for fixed effects from multi-level multivariable logistic regression models for prevalence of *E. coli* O157, *Salmonella*, and non-O157 STEC for sample types.

Sample types	Fixed effects	*Salmonella*		*E. coli* O157:H7		Non-O157 STEC	
		OR	95% CI	OR	95% CI	OR	95% CI
Feces							
Season	Rainy**						
	Dry	0.59	0.33–1.08	0.45	0.20–1.00	0.47	0.30–0.74*
Sex	Steer**						
	Cow	0.71	0.33–1.53	2.03	0.91–4.55	0.64	0.37–1.12
	Age	0.72	0.47–1.11	0.79	0.50–1.27	1.03	0.79–1.34
Hides							
Season	Rainy**						
	Dry	1.08	0.46–2.52	1.17	0.36–3.76		
Sex	Steer**	Baseline		Baseline			
	Cow	3.24	1.20–8.74*	1.73	0.41–7.28		
	Age	0.55	0.32–0.93*	0.46	0.21–1.11		
Carcasses							
Season	Rainy**						
	Dry	5.90	1.18–29.57*	1.58	0.30–8.25		
Sex	Steer**						
	Cow[1]	0.49	0.06–4.21	0.42	0.03–5.81		
	Age	0.56	0.19–1.70	0.98	0.27–3.58		

* $P < 0.05$.
** Baseline data, used as the variable to compare with the other within the same group.

but it was only significant for the detection of *Salmonella* from hides (OR = 0.55, 95% CI 0.32–0.93).

4. Discussion

This is the first comprehensive baseline research available to the public, that provides scientific information about *Salmonella*, *E. coli* O157:H7, and STEC non-O157 prevalence in the beef production chain in Colombia. Seasonal effect, sample type, sex and age of the animals were explored in relation to pathogen prevalence. The sampling was comprehensive, including the five largest abattoirs of the country, which not only slaughter about 50% of the beef cattle in the country, but also receive animals from all the major producing areas in Colombia (Figure 1).

In general, the proportion of *Salmonella* and *E. coli* O157:H7 on hides and carcasses had a tendency to be higher during dry months compared to rainy season, contrary to fecal samples. Findings of this study show that season appears to affect the occurrence of STEC O157 and non-O157 in fecal samples, in which a higher prevalence was observed during rainy compared to dry months; however, this was not the case for *Salmonella*. The seasonal effect on beef pathogen carriage is widely known and has been studied and reported elsewhere. A study conducted in the US found a higher prevalence of *E. coli* O157 and *Salmonella* during warmer than colder months (Barkocy et al., 2003). Contrary to that, in Scotland researchers found a higher prevalence of *E. coli* O157 during colder months compared to warmer months; although, they found that the concentration of *E. coli* O157 shed in feces was higher during warmer months (Ogden et al., 2004). Researchers attribute this phenomenon to the fact that animals are housed during winter, and animal to animal contamination may occur due to the close proximity to one another. In Argentina, a seasonal study was conducted by Fernandez et al. (2009) to investigate the prevalence of STEC based on the detection of *stx* genes in feces obtained from dairy cows. They found a *stx* prevalence of 22% in Fall, 28% in Winter, 56% in Summer, and 44% in Spring. During warmer months the shiga toxin encoding gene seemed to be appear at higher rates. The researchers also detected *E. coli* O157 during the four season and found an overall of 0.2% prevalence, much lower than other STEC. Non-O157 serogroups were not characterized in this research. Even though Argentina is a Latin American country, is located in the southernmost part of the continent posing a very different climate relative to other countries in the continent.

Seasonal studies have been conducted in locations where the four seasons (Summer, Fall, Winter, and Spring) occur, and unfortunately their results do not compare with seasonal prevalence in Colombia. This country has a tropical climate as is located in the northwestern part of South America, on the Equator line; therefore, distinct seasons are absent. In tropical countries, seasons are rather characterized based on rain influx rather than on temperature changes; thus, they recognize only rainy or dry season. In Colombia, precipitation is mainly related to El Niño and La Niña weather patterns; nonetheless, without the influence of these climate phenomena, typical rainy months are April, May, October, and November, while dry months are December, January, July, August.

Comparably to the present study, Chaves et al. (2015) found a similar trend and determined that in Costa Rica, a higher prevalence of STEC non-O157 occurs during rainy months than during dry months. It is possible that environmental conditions during rainy or wet days increase the likelihood of bacterial multiplication in the environment, which could be acquired by the animals and increase pathogen shedding. Interestingly, the present research found the opposite effect on hides with a lower prevalence during rainy months, could be associated to the cattle being cleaner as they are washed with the rain at the holding pens.

Irrespective of the weather, it was observed that hides presented a higher prevalence of *Salmonella* (15.0%) and *E. coli* O157:H7 (6.8%) compared to feces (8.8% and 4.8%, respectively). This appear to be a common issue in other slaughtering plants, and it represents a food safety concern. Animals presented for slaughter that carry pathogens on their hides, will become a source of contamination inside the facilities during carcass dressing. Findings in this investigation are consistent with research conducted in various countries, where a similar trend has been observed (Barkocy et al., 2003; Bosilevac et al., 2015; Chaves et al., 2015; Narvaez-Bravo et al., 2013a, b). Hides most likely get contaminated with feces during animal grazing, transportation, and at holding pens. The source of feces could potentially be from the animals themselves, during lying, or by animal to animal contact during transportation and holding pens. These results suggest that abattoirs must pay special attention to carcass dressing practices and prevention of cross-contamination from hides to food-contact surfaces, workers, or other carcasses. Quiguanas et al. (2020) conducted a study in Colombia in which only 21 animals were included from one single farm located in the Southwest of the country. Their PCR analysis identified that 57.1% of the animals were STEC positive based on the sxt encoding gene. Although this was a rather small sample, it has a great significance since it shows the high frequency at which the STEC virulence factors can be found in beef cattle feces.

Not surprisingly, post-intervention chilled carcass presented the lowest pathogen incidence since they underwent antimicrobial treatments. The prevalence of *Salmonella* on carcasses was 6.8% and 1.96% during dry and rainy season, respectively (4.4% overall). For *E. coli* O157:H7 the prevalence was 2.91% and 1.96% during dry and rainy months, respectively (overall 2.4%). When compared with hides, we observed a significant pathogen reduction on carcasses in both seasons. Piedrahita et al. (2001) evaluated beef carcass collected from two slaughterhouses located in the north of Colombia. Similar to the present study, 2% of the samples were found to be positive for *E. coli* O157:H7. Conducting sanitary dressing, following hygiene practices, and applying antimicrobial interventions are required measures to control bacterial contamination on carcasses. Furthermore, pathogen findings on post-intervention chilled carcasses, is an indication of cross-contamination. During the slaughtering process, bacterial pathogens present on hides, should not be transferred to carcass unless there is a failure during dressing, evisceration, and processing practices. Workers, food contact surfaces, and utensils could be a source of contamination if sanitation practices are not followed appropriately. Similar studies conducted in US slaughtering facilities as well as in other countries have also reported some pathogen incidence on post-intervention carcasses. In the United States, Barkocy et al. (2003) found the prevalence of *Salmonella* and *E. coli* O157 to be 0.1% and 1.2%, respectively on 1,232 post-intervention carcasses sampled. Similarly, Rivera-Betancourt et al. (2004) sampled carcasses post-intervention in two different slaughter houses in the US; the research group found a *Salmonella* prevalence of 0.0 and 0.8% in plants A and B, respectively; similar results for *E. coli* O157:H7 were observed with values of 0.0% in plant A and 0.1% in plant B. The national microbiological baseline conducted by USDA FSIS during 2014 and 2015 (USDA, 2015a, b, c), revealed on pre-chill beef carcasses after interventions, an overall prevalence of 3.36% for *Salmonella* and 0.66% for *E. coli* O157:H7. In Northern Ireland, Madden et al. (2001) found 1.5% *Salmonella* prevalence (n = 200) and 0% *E. coli* O157:H7 (n = 780). In Argentina, Masana et al. (2010) reported an *E. coli* O157:H7/NM prevalence of 2.6%, in carcasses post intervention at beef exporting abattoirs; however, they did not test for *Salmonella*. In Mexico, Narvaez-Bravo et al. (2013a, b) found a much higher incidence on *Salmonella* in post-intervention carcasses at 6.0%, while *E. coli* O157:H7 was 0.4%. Their study found consistent results in feces, where animals were higher in *Salmonella* than *E. coli* O157:H7.

In Colombia, slaughterhouses testing plans and government surveillance tend to focus their efforts primarily on *E coli* O157:H7 while a lower testing frequency for STEC non-O157 and *Salmonella* is conducted. This is perhaps based on the assumption that there is a lower prevalence of these pathogenic groups in their beef cattle. One of the major contributions to the present study, was to report that the "big six" non-O157 STEC had the highest prevalence in fecal samples (51.0%), compared to *E. coli* O157:H7 (5.0%) and *Salmonella* (8.3%). The most prevalent STEC serogroup was O45 (57.2%, 99/173), followed by O121 (40.5%, 70/

173), O103 (31.2%, 54/173), O26 (20.2%, 35/173), O145 (15.0%, 26/173), and O111 (4.0%, 7/173). It should be noted that 84.7% of the fecal samples analyzed carried at least one of the virulent genes associated to STEC infections (*stx* or *eae*). This finding suggests that in Colombia testing for STEC non-O157 should be a priority. In the US, STEC prevalence of 19.4% in and 18.4% in bovine feces has been found in some studies (Barkocy et al., 2003; Samadpour et al., 2002). Argentina reported 69% of fecal samples carrying the *stx* gene (Meichtri et al., 2004). Other studies worldwide also indicate that STEC presence in beef carcasses can be as high as 18% in Calcutta, India (Khan et al., 2002), and 26% in Australia (Barlow and Mellor, 2010). The big six STEC non-O157 are responsible for 74% of non-O157 STEC infections in the United States, based on the U.S. Centers for Disease Control and Prevention (Scallan et al., 2011). *Salmonella* on the other hand, seem to be present at lower concentrations in beef cattle. Reports indicate a prevalence of 6.2% in feces in the US (Rhoades et al., 2009), and 1.9% in Canada (Sorensen et al., 2002). In the US, *E. coli* O157:H7 has been found in feces at 28% (Elder et al., 2000), 7.5% (Omisakin et al., 2003), and 5.8% reported by Barcoky et al. (2003), who also found a prevalence as low as 0.3% during winter. In Ireland, fecal samples collected from an export-class abattoir over a period of 14 months showed a level of *E. coli* O157:H7 2.7% (Thomas et al., 2012).

This investigation found evidence of an association between the age of the animal and pathogen prevalence, identifying less frequency of *E. coli* O157:H7 and *Salmonella* as the age of the animal increased. Similar results are reported by Cray and Moon (1995), who found that fecal shedding of *E. coli* O157:H7 had higher prevalence levels in calves than in adults. The authors suggest that this can be due to a more developed stomach in which high volatile fatty acids suppress the growth of *E. coli* O157:H7 (Cray and Moon, 1995). Contrary to these results, Mir et al. (2015) found an inverse influence between animal age and STEC shedding in feces. Based on their study, heifers have an STEC prevalence of 37.5% while cows 70%. Due to variation in reports, it is no feasible to provide conclusions in this regard. It should also be mentioned that based on data collected during sampling, animals presented for slaughter in Colombia could be up to 6 years old; the age distribution of the animals sampled in this project was 16.2% animals of 1–2 years of age, 77.7% between 3-4 years, and 6.1% between 5-6 years old.

Protein of animal origin locally produced in low income countries are an important component of their diet. Therefore, control of foodborne pathogens must be a priority to improve public health and increase the quality of life in developing countries. A higher pathogen prevalence on carcasses, as compared with data obtained in developed countries, could be addressed with intensified hygiene and sanitation practices. On the other hand, reduction of pathogen carriage by food-animal must be addressed. The concept of "One Health Approach" should be considered in the design of mitigation strategies, where scientists, university experts, physicians, veterinarians, cattle farms owners, processing companies' leaders, and government official work together in find solutions, set common objectives and desig strategies (Torres, 2017).

This paper is the first scientific research published to show the significance of the foodborne pathogens presence such as STEC O157, non-O157, and *Salmonella* in the Colombian beef production chain. It is of the greatest importance to increase national surveillance of these foodborne pathogens, make data available to the public, correlated surveillance data with illnesses and outbreaks. When stakeholders work together towards the same public health objective, the design and implementation of control measures could be more effective.

Declarations

Author contribution statement

Alexandra Calle: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ana Karina Carrascal: Conceived and designed the experiments; Performed the experiments.

Carlos Patiño, Alejandro Echeverry, Mindy Brashears: Contributed reagents, materials, analysis tools or data.

Carlos Carpio: Analyzed and interpreted the data.

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Data included in article/supp. material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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