



Evaluation of Process Control to Prevent Contamination of Beef with Non-0157 Shiga Toxin-producing *Escherichia coli* (STEC) in U.S. Export Abattoirs in Honduras and Nicaragua

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ABSTRACT

The objectives of this project were to determine (1) the prevalence of non-0157 STEC on beef hides and carcasses in export abattoirs in Honduras (Plant A) and Nicaragua (Plant B) and (2) whether current practices and interventions controlled final carcass contamination. Samples were collected on the foreshanks from the hides, at pre-evisceration and after application of an antimicrobial treatment. In Plant A, 23.3% (7/30) of hides contained at least one STEC serogroup, whereas in Plant B, 90.0% (45/50) tested positive. Pre-evisceration samples had a prevalence of 6.7% (2/30) for Plant A and of 0% for Plant B. No STEC were detected after antimicrobial intervention. Serogroups 026, 045, and 0121 were the most prevalent in plant A, with frequencies of 27/75 (36.0%), 24/75 (32.0%), and 18/75 (24.0%), respectively. In Plant B, 026 and 0121 were predominant, with 47.5% (38/80) and 46.3% (37/80), respectively. STEC were present on the hides, but current hygienic practices and interventions effectively controlled them and reduced final carcass contamination.

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INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) have become a food safety problem over the past few decades because of their association with major foodborne disease outbreaks (9, 16). Although *E. coli* O157:H7 is the most frequent serotype associated with such outbreaks in the U.S., more than 100 different STEC serotypes have been related to human disease globally (1, 6, 11, 13, 20). Worldwide, a recent increase in the number of non-O157 STEC infections has been reported, which has led government and health agencies to focus their attention on these serotypes as a group of emerging pathogens of concern (12, 18, 19, 24).

In addition to *E. coli* O157:H7, six major O groups are most commonly associated with illness in the U.S.: O26, O45, O103, O111, O121, and O145 (hereafter referred as the “big six”), and these six O groups, comprising 13 serotypes, cause a wide spectrum of human diseases, ranging from mild diarrhea to hemorrhagic colitis (HC) and the life-threatening hemolytic uremic syndrome (HUS) (3, 9). The U.S. Centers for Disease Control and Prevention (CDC) have described these six O groups as the cause of 71.0% of non-O157 STEC diseases in the U.S. However, information is lacking on their epidemiology, clinical spectrum of illness, and modes of transmission, compared with O157:H7 (8, 19).

Cattle are one of the major reservoirs of *E. coli* O157:H7 and non-O157 STEC and the main source of contamination of the human food supply (1, 4, 11). STEC, as a normal part of the microflora of their gastrointestinal tract, can contaminate meat and slaughterhouse environments. Consumption of food and water contaminated with feces and direct contact with animal feces are primary routes of human infection (11, 12, 20, 26, 27).

During beef processing, feces and beef hides are significant sources of carcass contamination (1, 20). At the point of slaughter, numerous opportunities for non-O157 STEC contamination and cross contamination arise. Studies have shown the prevalence rates in whole beef carcasses of STEC O157 and non-O157 ranged from 0.01 to 43.4%, and 1.7 to 58.0%, respectively (10, 11). However, good dressing procedures and antimicrobial interventions (e.g., organic acids) in beef processing environments are known to significantly reduce the number of bacteria of fecal origin on beef carcasses (20, 27).

In 2012, the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) declared six new STEC serotypes as adulterants in raw ground beef (25). This standard applies to beef produced in the U.S. as well as imported beef. The plants producing beef must meet the standards set forth by the FSIS with regard to control of STEC in their process. Therefore, it is essential to validate the process controls in facilities that export beef to the U.S. in order for them to provide a safe product as well as to maintain the export market. The objectives of this project were to determine the prevalence of non-O157 STEC on beef hides and beef carcasses at different production stages in two abattoirs with implemented Hazard Analysis Critical Control Points (HACCP) food safety management systems in Honduras and Nicaragua and to determine whether current dressing procedures and interventions were effective in controlling beef carcass contamination.

MATERIALS AND METHODS

Location and abattoir description

Two beef abattoirs were visited in April or May of 2012. One plant is located near Tegucigalpa, Honduras (Plant A) and the other near Managua, Nicaragua (Plant B). Both plants have implemented HACCP as their food safety management system and have been cleared for export of whole beef carcasses and raw beef cuts to the U.S. Combined, the two facilities export more than 6.3 million pounds of beef product to the U.S. annually and have no previous history of problems with regard to food safety. Because of their export status, they are inspected at least once a year by the USDA-FSIS as part of the equivalence evaluation process for imported meat. HACCP records are kept and both plants test beef trim for *E. coli* O157:H7, using the DuPont Qualicon BAX system[®]. According to the general managers, beef cattle are brought in from different regions of each country and are predominantly grass-fed. On average, 180 beef cattle are harvested per day, with the plant operating 6 days a week. In both plants, the antimicrobial intervention consists of a 2–2.5% lactic acid spray after final beef carcass inspection prior to chilling.

Sample collection

Individual carcasses were tagged and followed along the processing line and sampled at various points. Sterile sponges pre-hydrated with 10 ml of neutralizing buffered peptone solution (World Bioproducts; Mundelein, IL, USA) were used to swab the fore Shank of the carcasses immediately before skinning (hide samples), prior to evisceration (pre-evisceration samples, i.e., after complete hide removal), and directly after application of the antimicrobial treatment (post-intervention) before the carcass entered the hot box. In Plant A, 30 swabs were collected at each point for a total of 90 samples, corresponding to 30 animals. Similarly, 50 swabs were collected at each point in Plant B for a total of 150, corresponding to 50 animals. Sample collection was conducted throughout the day to reduce the possibility of sampling clusters of unusually contaminated carcasses. Sponges were kept refrigerated and then transported to Texas Tech University in cooler bags with frozen coolant packs via commercial airline within 24 hours of sample collection. USDA's Animal and Plant Health Inspection Service (APHIS) permits were obtained in advance to bring samples back to the U.S.

Detection of non-O157 STEC

Upon arrival at the laboratory, sponges were manually massaged for 30 seconds and 1 ml of the solution expressed was transferred to 9 ml of 0.1% wt/vol Buffer Peptone Water (BPW; Becton Dickinson, Sparks, MD) for enrichment at $42 \pm 1^\circ\text{C}$ for 18 h. An FSIS-approved real-time PCR protocol was used to assess the presence of the “big six” non-O157 STEC (O26, O45, O103, O111, O121, O145) on all sponges, using the DuPont Qualicon BAX[®] for screening of *stx* and *eae* genes and final confirmation of the O antigen (panels 1 and 2, see below). A sample was considered positive for non-O157 STEC if both genes were present. Cycle threshold (Ct) values between 0 and 43 were considered positive as per equipment instructions for all three reactions: screening, panel 1 and panel 2. A cocktail of the “big six” non-O157 STEC of bovine origin was used as internal positive control and ultrapure

water was used as blank. Manufacturer's instructions were followed for the PCR screening reaction. Briefly, the lysis reagent was prepared by adding 150 μ l of protease to one 12-ml bottle of lysis buffer. After gentle mixing, 200 μ l of lysis reagent was placed in cluster tubes and 20 μ l of the enriched samples was added to the corresponding tubes for each sample. Cluster tubes were heated in pre-warmed blocks, first at 37°C for 20 min and then at 95°C for 10 min. After lysis completion, cluster tubes were inserted into a cooling block chilled at 2–8°C for at least 5 min. Subsequently, 50 μ l of lysate was transferred to clear tubes with optical caps containing all the PCR reagents in a tablet and taken chilled to the PCR device. Software instructions were followed and readings were obtained after 60 min. Samples deemed positive after screening for *stx/eah* genes were subjected to a second PCR reaction to determine the O antigen by means of Panel 1 (for O26, O111, and O121) and Panel 2 (for O45, O103, and O145). For this purpose, 50 μ l of lysate was transferred to clear tubes with optical caps containing all the corresponding PCR reagents in a tablet and taken chilled to the PCR device. Software instructions were followed and readings were obtained after 60 min.

Data analysis

Absolute and relative frequencies of positive non-0157 STEC samples were determined and 95% confidence intervals for proportions were estimated using the Statistical Analysis System, SAS, version 9.3 (SAS Institute, Cary, NC). Furthermore, Fisher's exact test, estimated via PROC FREQ on SAS, was used to determine differences between plants for the proportion of positive samples at each sampling location. Differences were deemed significant at a 5% probability level.

RESULTS

Prevalence of non-0157 STEC on beef hides and carcasses

Eighty beef carcasses were surveyed for the presence of the "big six" non-0157 Shiga toxin-producing *E. coli* (STEC), for a total of 240 sponge samples in both plants. In Plant A, 23.3% (7/30) of the hide samples contained at least one STEC serogroup, whereas in Plant B, 90.0% (45/50) of the samples taken on the hides at pre-skinning tested positive. Statistically, the prevalence was significantly higher in hides in Plant B than in those in Plant A (**Table 1**). Samples taken at the subsequent sampling point, pre-evisceration, showed lower prevalence: 6.7% (2/30) for Plant A and no STEC detected at Plant B. Similarly, no STEC were detected after application of the antimicrobial treatment (post-intervention) on any of the samples taken at either plant (**Table 1**). No statistical differences were observed between prevalence at pre- or post-evisceration in either plant (**Table 1**).

Non-0157 STEC serogroups distribution

A total of 75 molecular markers for non-0157 STEC "big six" were detected from a total of 9 positive samples from Plant A, whereas 80 were detected from a total of 45 positive samples collected in Plant B. Serogroups O26, O45, and O121 were the most prevalent in Plant A, with frequencies of 27/75 (36.0%), 24/75 (32.0%), and 18/75 (24.0%), respectively. Similarly, in Plant B, serogroup O26 was the predominant one, with 47.5% (38/80), but was very closely followed by O121, with 46.3% (37/80). In both facilities, serogroups O103 and O145 were detected in much smaller proportions, while serogroup O111 was not

TABLE 1. Prevalence of non-0157 on beef carcasses in abattoirs in Honduras (Plant A) and Nicaragua (Plant B) based on sampling location

SAMPLING LOCATION	PLANT A – HONDURAS		PLANT B – NICARAGUA	
	NO. POSITIVES/NO. TESTED (%)	95% CI ¹	NO. POSITIVES/NO. TESTED (%)	95% CI ¹
HIDES	7/30 (23.3%) ^a	11.9–41.1	45/50 (90%) ^b	78.6–95.6
PRE-EVISCERATION	2/30 (6.7%) ^a	2.0–21.4	0/50 (0.0%) ^a	0.0–7.0
POST-INTERVENTION	0/30 (0.0%) ^a	0.0–11.2	0/50 (0.0%) ^a	0.0–7.0

¹95 CI, 95% Confidence Interval

^{a,b}Values with the same letter in a row are not significantly different at a significance level of 0.05

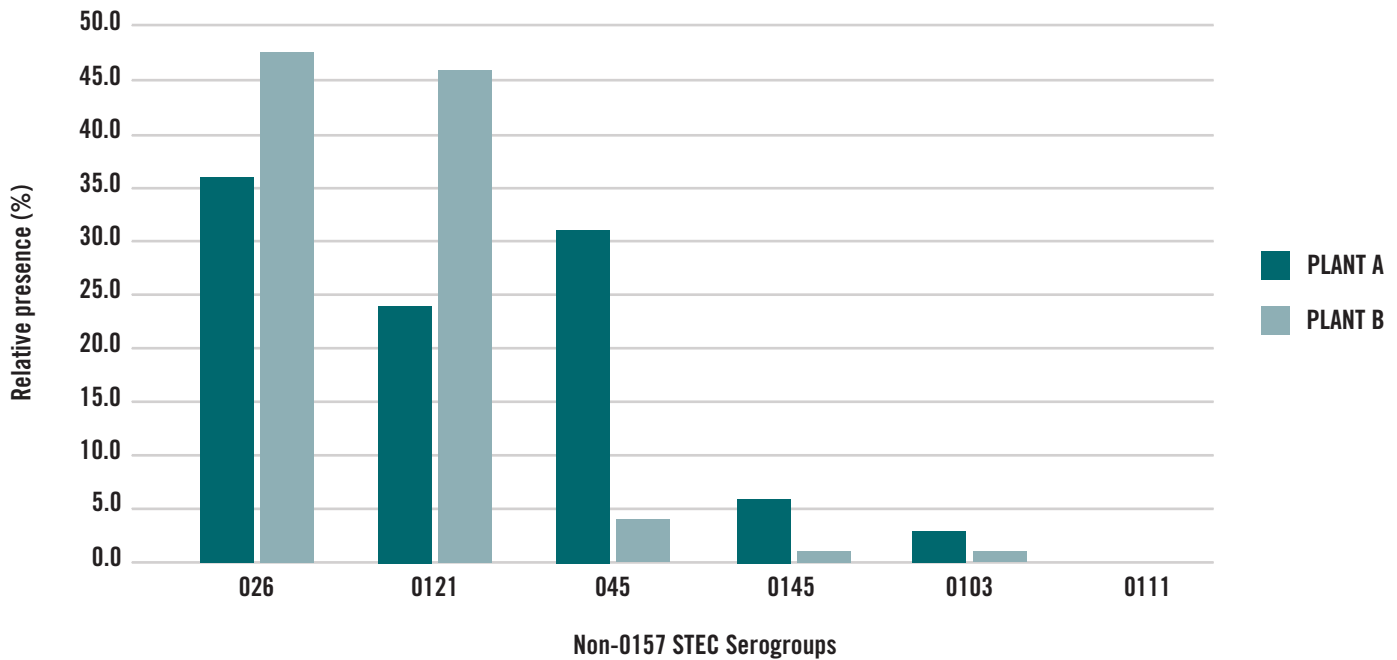


Figure 1. Prevalence (%) of non-0157 STEC serogroups screened for on beef hides sampled immediately before skinning (hide samples) in two abattoirs in Honduras (Plant A) and Nicaragua (Plant B)

detected in any sample at either abattoir (Fig. 1). Additionally, two of the 30 pre-evisceration samples (6.7%) taken in Plant A tested positive for non-0157 STEC, with serogroups 026, 045, and 0103 being present in both samples.

DISCUSSION

Hides of beef cattle presented for slaughter have been shown to be a major source of pathogenic bacteria in processing plants (5, 10). It is, therefore, expected that this type of sample had the highest prevalence of non-0157 STEC of the three points evaluated in this study. The prevalence was significantly ($P < 0.05$) lower in Plant A (23.3%) than in Plant B (90.0%); however, both values are in accordance with previous studies that reported a broad range of prevalence for samples collected on beef hides. In a study conducted at four large processing plants in the U.S., Arthur et al. (1) found pre-evisceration prevalence of non-0157 STEC to be 54.0% (180/334). Of the 30 lots researchers used to collect pre-evisceration samples, 28 (93.3%) were positive for non-0157 STEC, and the prevalence of positive pre-evisceration samples within a lot ranged from 0 to 100%. Furthermore, in an investigation of seasonal prevalence of *E. coli* 0157, *Salmonella*, non-0157 STEC, and *stx*-harboring cells at three Midwestern fed-beef processing plants, Barkocy-Gallagher et al. (4) found non-0157 STEC hides prevalence peaked at 77.7% (258/332 samples) in the fall, while summer and fall prevalence remained above 85.0% throughout all four seasons. Several reasons may account for the variations in the reported values for prevalence of non-0157 STEC on hide samples. For instance, feces from one animal can contaminate multiple hides, and hides can be contaminated with feces from multiple animals; therefore, samples

may reflect both lot and individual contamination (4). Lairage has also been proposed as a source of pathogens on beef hides, at least for *E. coli* 0157, and presumably for other serotypes. According to Arthur et al. (2), transportation of beef cattle to lairage at processing plants can lead to an increase in prevalence and extent of *E. coli* 0157:H7 on beef hides. The authors hypothesized that during the holding pens' wash procedure at night, holding cattle are most likely to come in contact with the spray and runoff when adjacent pens are washed down. This contact may account for the presence of bacteria of different types on beef hides in a short period of time (2).

The second sampling stage evaluated in this study was pre-evisceration. Sampling at this point serves to measure pathogen transfer from hides to carcasses. At this step in production, immediately following hide removal but before the pre-evisceration wash, carcasses are subjected to no antimicrobial interventions. Results of this survey showed very few samples were contaminated with non-0157 STEC at this step. In Plant A, 6.7% (2/30) were found to harbor at least one of the non-0157 STEC serogroups screened for, while none were detected in samples from Plant B. The difference was non-significant ($P > 0.05$). This low prevalence indicates that employees were following good hygienic practices for carcass dressing. Much higher prevalence values have been detected elsewhere. For example, in the U.S., Barkocy-Gallagher et al. (4) found at least 90.0% of pre-evisceration carcass samples were *stx*-positive throughout a one-year period, with an overall prevalence of 96.5%. Rigobelo et al. (21) evaluated the presence of STEC on beef carcasses in an abattoir in Southwestern Brazil and found a 20% (120/600) prevalence of carcasses carrying *stx* genes just prior to evisceration, as determined by PCR.

One primary indicator of beef-product safety is the prevalence of carcass contamination after the application of an antimicrobial intervention (post-intervention) (4). In addition, pathogen presence on post-intervention beef carcasses is significant to public health issues because consumer risk increases as the product becomes case ready. Samples taken from dressed carcasses were deemed PCR-negative for the presence of the six non-O157 STEC serogroups screened for. No significant differences were detected between plants for prevalence at this production step, as expected. Actual numbers may range from 0 to about 11% in Plant A and from 0 to 7% in Plant B, based on the estimated 95% confidence intervals. Our results are in agreement with those of Rogerie et al. (23), who reported a post-processing prevalence of non-O157 STEC of 1.9% on carcasses sampled after chilling at processing plants in France. Similarly, the non-O157 STEC prevalence on carcasses processed in Hong Kong was reported to be 1.7% by Leung et al (17). Rigobelo, Santo, & Marin (22) found a prevalence of 1.3% (3/216) positive *stx*-harboring carcasses in a survey in a small abattoir in Southwestern Brazil, presumably studying samples collected after antimicrobial interventions had been applied.

In both abattoirs visited in this study, the final antimicrobial intervention was a carcass spray with a 2–2.5% lactic acid solution, demonstrating the effectiveness of this intervention in reducing the numbers of non-O157 STEC on the surfaces of beef carcasses. In another investigation, Shiga toxin genes were detected in 200 (16.2%) of 1,232 post-intervention carcass sampled by Barkocy-Gallagher et al. (4) and a prevalence of 12.3% of *stx*-carrying carcasses was reported by Etcheverría et al. (11) on beef carcasses at storage in two Argentinean slaughterhouses in Buenos Aires province. Another study, conducted in Argentina by Masana et al. (18), showed 73 (9.0%) of 181 carcass swabs taken before entering chilling rooms were positive for non-O157 STEC contamination, as determined by PCR. Interestingly, the abattoirs that participated in the study did not apply any sort of microbial decontamination strategy. Values for post-intervention prevalence for this study are within the ranges reported elsewhere in the literature.

Comparisons among studies should be made cautiously, as samples have been collected in different regions of the world with varying feed regimens and in different seasons (14). The real-time PCR methodology used in this study allows the detection of *stx* and *eae* genes if present. This method is preferred to isolation, as it has been proven that plating can underestimate the prevalence of non-O157 STEC, possibly because of lack of adequate culture methods (4). Multiple other variables may cause differences in results, including management practices and animal factors such as age, sex, and breed (14). Given the real-time PCR methodology employed here, all samples deemed positive for non-O157 STEC carried not only one or both *stx* genes but also the *eae* gene partly responsible for attaching and effacing lesions of the host cells. Although it has been reported that some *eae*-negative non-O157 STEC strains are able to produce HUS, most disease-implicated serovars contain the *eae* gene (15).

The screening for the so-called “big six” non-O157 STEC (O26, O45, O103, O111, O121, and O145) is a response to their recent classification as adulterants in non-intact meat and ground beef products by the USDA. The results of this study revealed that serogroups O26, O45, and O121 were the most prevalent of the six evaluated and accounted for over 90% of the non-O157 STEC detected in the two plants. Serogroups O121 and O145 represented very small proportions of the total number of non-O157 STEC identified by real-time PCR and no O111 was detected. One potential factor influencing occurrence of different serogroups is the natural presence of diverse non-O157 varieties in soils. Bolton et al. (7) demonstrated that non-O157 STEC occur widely and frequently in pasture soils in Ireland and can persist in such environments for several months, with considerable opportunity for recycling through farm environments and cattle.

Few reports of non-O157 STEC infections in Latin America are found in the literature, except for Argentina, where an estimated 30.0% of post-enteric HUS cases are caused by non-O157 STEC strains. Masana et al. (18) reported data collected by the National Reference Laboratory for HUS surveillance of Argentina showing that in a 5-year period (2004 to 2009), 1,066 STEC-associated infections were reported, among which the more prevalent non-O157 STEC O-groups were O145 (17.5%), O121 (2.4%), O26 (2.0%), O174 (1.4%), O111 (1.0%), and O103 (0.7%).

CONCLUSIONS

The prevalence of non-O157 STEC was high on hides of beef cattle entering the abattoirs. However, proper dressing procedures, together with effective antimicrobial interventions, reduced STEC contamination on final carcasses. Results demonstrate the effectiveness of spraying carcasses with a 2–2.5% lactic acid solution prior to chilling in reducing the prevalence of non-O157 STEC and highlight the significance of hides as a major source of these pathogens on carcasses. To the authors’ knowledge, the current study is the first of its kind. Although all known STEC serogroups and virulence genotype populations associated with cattle were not studied in this investigation, the results may serve as a first step in characterizing microbial contamination of beef hides and carcasses in Honduras and Nicaragua. They can also aid in assessing risk, attributing non-O157 STEC illness to beef products, and validating process control.

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