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Research Note

Occurrence of the Seven Most Common Serotypes of Shiga Toxin–Producing *Escherichia coli* in Beef Cuts Produced in Meat Processing Plants in the State of São Paulo, Brazil

MIRIAM GONÇALVES MARQUEZINI *https://orcid.org/0000-0002-3577-4423*,[1] **LUIS HENRIQUE DA COSTA**,[2] **AND RENATA BROMBERG** *https://orcid.org/0000-0002-0817-2144*[1]*

[1]*Meat Technology Center - CTC, Institute of Food Technology, Avenida Brasil 2880, CEP 13070-178, Campinas, São Paulo, Brazil; and* [2]*Merck S. A. Brazil, Alameda Xingu 350, 7° andar, CEP 06455-030, Barueri, São Paulo, Brazil*

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ABSTRACT

Healthy cattle are considered the main reservoir of Shiga toxin–producing *Escherichia coli* (STEC) strains, so in some places in the world, products derived from beef are the most common source for disease outbreaks caused by these bacteria. Therefore, to guarantee that the beef produced by our slaughterhouses is safe, there is a need for continuous monitoring of these bacteria. In this study, 215 beef cuts were evaluated, including chilled vacuum-packed striploins (151 samples), rib eyes (30 samples), and knuckles (34 samples), from March to June 2018. These meat samples were collected from the slaughter of unconfined cattle, being arbitrarily collected from eight meat processing companies in São Paulo state, Brazil. Each sample was examined for the presence of STEC toxin type (stx_1 and/or stx_2 genes) and also the attaching and effacing *E. coli* (*eae*) gene, determined by a multiplex PCR assay. We show that the major seven STEC strains (O serogroups O26, O45, O103, O111, O121, O145, and O157) are not detected in any of the analyzed beef cut samples; however, three of them presented the virulence *eae* gene. Therefore, the absence of STEC strains in the beef samples may be an indication of the low prevalence of this pathogen in the cattle herd on the farm, associated with good hygiene and handling practices adopted by the meat industry.

HIGHLIGHTS

- No STEC strains were detected by multiplex PCR assay in 215 beef samples.
- Gene *eae* was identified in three (1.4%) STEC isolates in striploin samples.
- The type of bovine cut analyzed did not influence the occurrence of STEC.

Key words: Beef; *Escherichia coli*; Foodborne pathogens; Shiga toxin; Shiga toxin–producing *Escherichia coli*

Shiga toxin–producing *Escherichia coli* (STEC) constitutes a large group of enteric pathogenic bacteria that have acquired genes responsible for causing intestinal or extraintestinal diseases *(25),* considered to be the main cause of bloody diarrhea and hemolytic uremic syndrome. This condition causes hemolytic anemia, thrombocytopenia, and potentially fatal acute renal failure. *E. coli* O157:H7 was the first isolated STEC serotype associated with outbreaks of foodborne disease due to the consumption of undercooked beef in restaurant chains *(29)*. Strains of different serotypes, such as O26, O45, O103, O111, O121, and O145, have also been recognized for causing severe disease and are currently described as the "top six" non-O157 STEC *(8, 39)*.

The main virulence factor for STEC, which is also referred to as enterohemorrhagic *E. coli* (EHEC), is the production of Shiga toxins (Stx) that includes two major forms: Stx_1 and Stx_2. The stx_1 and stx_2 genes located in bacteriophage genomes, integrated into the bacterial host genome, encode the Shiga toxin production and, therefore, are under the control of phage genes *(13)*. Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation *(2)*. In addition to Shiga toxins, most STEC strains possess the locus of enterocyte effacement pathogenicity island, where the *eae* virulence gene, which encodes the intimin adhesion factor, is located *(27)*.

Although STEC O157 and non-O157 strains have been isolated from the guts of a variety of domestic and wild animals, cattle are considered the major reservoir host of these pathogens *(3)*. STEC may be transmitted to humans by the consumption of foods or water contaminated with

* Author for correspondence. Tel: (55 + 19) 3743-1880; Fax: (55 + 19) 3743-1882; E-mail: renatab@ital.sp.gov.br.

cattle manure, direct contact with animals, person-to-person contact, use of recreational contaminated water, as well as through infection caused by rodents and insects *(18, 31)*. Therefore, water sources contaminated with ruminant feces can be vehicles of STEC infection, through the cross-contamination of food, water, and the environment. In fact, any food category may contain STEC, including meat and meat products, fruits, unpasteurized fruit juices, salads, sprouts, and milk *(35)*. The prevalence of *E. coli* O157 in cattle varies considerably between countries, depending on the season, sampling strategy, detection method, and geographical location *(19, 32)*. In addition, cross-contamination with STEC in slaughterhouses and retail results in a possible contamination of meat and meat products at points of sale *(19)*. One of the most common causes of STEC infection is the consumption of raw or undercooked foods of bovine origin, particularly hamburgers *(27, 33)*. The mincing process evenly distributes the STEC population present on the surface of the meat, but if hamburgers and meatballs are not fully cooked, the bacteria placed in the central portion may not be exposed to lethal temperatures *(27)*.

Export figures show Brazil's position as one of the main beef producer and exporter countries in the world *(41)*. According to the Brazilian Institute of Geography and Statistics, the state of São Paulo was responsible for 20.2% of cattle slaughter in the country in 2018, occupying the first place in the national ranking *(21)*. Therefore, it must meet the quality and safety requirements demanded by national and international markets. Thus, it is necessary to keep its products free of pathogens, including those from the STEC group. To maintain the recognition of the quality of Brazilian beef, as well as the confidence in national production and competitiveness standards, the meat industry has intensively directed efforts to ensure the safety of the products. Hence, this study aimed to verify the occurrence of STEC in beef cuts samples from different meat companies in São Paulo state, Brazil.

MATERIALS AND METHODS

Samples. Beef products (215 samples) were evaluated individually, including cuts of chilled vacuum-packed striploins (151 samples), rib eyes (30 samples), and knuckles (34 samples), from March to June 2018. Each sample (approximately 1.0 to 1.5 kg) included about five pieces of beef cuts from the same lot. According to the International Commission on Microbiological Specifications for Foods *(22),* a lot corresponds to the quantity of food or food units produced and handled under uniform conditions. For the detection of STEC strains, the preparation of the sample unit was based on the number of samples and kits available. For this reason, a pool of samples from the same lot was prepared, according to the International Organization for Standardization ISO/TS 13136 method *(23)*. The sampling plan was not based on a specific model, as the samples were arbitrarily collected in eight establishments, located in São Paulo state, Brazil, that slaughter unconfined cattle and/or process beef (Table 1). The establishments, which have also implemented food safety programs, such as the hazard analysis and critical control point (HACCP) program, are divided into three classes according to the daily slaughter capacity: small (P), slaughtering up to 500 cattle daily; medium (M), slaughtering between 500 and 800 cattle daily; and large (G), with daily slaughter of more than 800 cattle *(5)*. Samples were placed in thermal boxes containing ice packs and were transported to the microbiology laboratory of the Meat Technology Center of the Institute of Food Technology (Campinas, São Paulo, Brazil), where they were stored at 4°C (±1°C) until analyses were performed.

Beef samples enrichments. About 50 g of each sample was aseptically weighed into blender bags (Whirl-Pak, Nasco, Fort Atkinson, WI), and these portions were arbitrarily collected from the exterior surface of the whole cut samples, with a thickness of 2 to 3 mm. Each subsample was then homogenized in a stomacher (Seward Limited, Worthing, WS, UK) with 450 mL of preheated (42°C) modified EHEC media (mEHEC; BioControl Systems Inc., Bellevue, WA) for 1 min, and then aerobically incubated at 42°C (±1°C) for 16 to 18 h.

PCR screening for *eae* and *stx* genes and confirmation. Following sample preparation with immunomagnetic separation against the top seven O groups, the samples were analyzed with a single multiplex assay for the presence of *eae, stx*$_1$, and *stx*$_2$ genes. Assurance GDS (BioControl Systems Inc.) was used to detect the presence of top seven O groups of Shiga toxigenic *E. coli* belonging to O serogroups O26, O45, O103, O111, O121, O145, and O157 in meat product samples.

An aliquot of the mEHEC enrichment was tested simultaneously with the Assurance GDS Top 7 STEC (*eae*) Tq and Assurance GDS Shiga Toxin Genes (Top 7) Tq kits. The analyzed samples were reincubated at 42°C (±1°C) until the end of the tests, to be used for confirmation. According to the GDS protocol, if samples were positive for the *eae* gene and either of the *stx*$_1$ or *stx*$_2$ genes, they were considered presumptive positive for Shiga toxigenic *E. coli* belonging to O serogroups O26, O45, O103, O111, O121, O145, or O157. Any presumptive-positive sample was confirmed from the retained mEHEC enrichment via the U.S. Department of Agriculture and Food Safety and Inspection Service (USDA-FSIS) *Microbiology Laboratory Guidebook* (method 5B.01) for *E. coli* O157:H7 and for the other top six serogroups *(37)*.

RESULTS AND DISCUSSION

Considering the 215 beef samples tested for the presence of Shiga toxigenic *E. coli* serogroups O26, O45, O103, O111, O121, O145, or O157, none of them were identified as STEC (Table 1). One of the virulence factors, the *eae* gene, was detected in three of the striploin samples, while no toxin gene, *stx*$_1$ or *stx*$_2$, was detected in any of the samples studied. In this study, samples of striploins and rib eyes were provided by large plants, while knuckles were the only sample type from the medium and small processors. As the processors provided samples voluntarily, there is not an equivalent representation of sample types among the establishments.

The GDS system adopted in this study has a protocol that included the immunomagnetic separation technique for the isolation of the STEC top serogroups. The use of PickPen immunomagnetic separation greatly improves the performance of the immunomagnetic separation procedure and may be more compatible with several emerging pathogen detection technologies, allowing the rapid isolation of multiple target pathogens of a single sample *(26)*.

TABLE 1. *Screening of STEC strains in commercial samples of beef cuts*

Meat processing company		Beef sample	No. of samples	Top 7 Shiga toxins (sxt_1 and sxt_2) (%):		Top 7 STEC (*eae*) (%):	
Code	Size[a]			Positive	Negative	Positive	Negative
1	Large	Striploin	40	0	100	7.5	98
2	Large	Striploin	40	0	100	0	100
		Rib eye	30	0	100	0	100
3	Medium	Knuckle	10	0	100	0	100
4	Small	Knuckle	8	0	100	0	100
5	Small	Knuckle	8	0	100	0	100
6	Small	Knuckle	8	0	100	0	100
7	Large	Striploin	36	0	100	0	100
8	Large	Striploin	35	0	100	0	100
Total			215	0	100	1.4	98.6

[a] The size of the slaughterhouse was considered according to the slaughter of heads per day *(5)*. Small, up to 500; medium, 500 to 800; large, more than 800.

The interactions between the environment and commensal bacterial adaptation indicate that virulence factors in humans, including locus of enterocyte effacement or Shiga toxins, may provide mechanisms for the survival of these microorganisms in other hosts or in the environment and an adaptive tool to retain certain characteristics *(36)*. One possibility to explain the origin of the strains that contain the *eae* gene would be that it is a chromosomal gene and, in addition to STECs, other bacteria carry it. The *eae* gene was isolated as a necessary locus for the attaching and effacing activity of enteropathogenic *E. coli (24)*. According to Donnenberg et al. *(11),* the *eae* gene is necessary for intimate attachment of STEC in vivo, and EHEC and enteropathogenic *E. coli eae* genes are functionally homologous. Enteropathogenic *E. coli* belongs to a group of pathogenic bacteria that can cause attaching and effacing lesions on the surface of the host's intestinal epithelium, is a noninvasive organism, and does not produce enterotoxins. Besides enteropathogenic *E. coli* and STEC, there are other microorganisms that display attaching and effacing activity, such as *Hafnia alvei, Citrobacter freundii (1, 15, 30), Escherichia albertii,* and atypical *Shigella boydii (20)*. In a study carried out in The Netherlands, which analyzed a set of 209 STEC non-O157 in animal, meat, and human clinical isolates, the vast majority (80.9%) of the included STEC strains were *eae* negative *(14)*. To be classified as STEC, strains must have detectable stx_1 and/or stx_2 genes. However, any STEC strain with *stx* and *eae* or other STEC adherence genes should be considered potentially pathogenic *(34)*.

Note that mobile elements that carry virulence factors present in these bacteria can be obtained or lost over time. According to Croxen et al. *(10),* to provide new traits, mobile genetic elements, including transposons, insertion sequences, bacteriophages, and plasmids, can integrate into the chromosome or self-replicate in the new host. Although new genes acquired through horizontal gene transmission provide bacteria with a variety of new traits, gene loss can also favor the fitness or adaptation of a pathogen in a particular niche. In addition, Shiga toxin genes can also be located in the genome of temperate phages found in food samples as free particles *(28)*.

In Brazil, although no STEC-related human disease outbreak has been reported, some STEC infections have been related to sporadic cases of nonbloody diarrhea caused by these strains *(7, 17)*. Nevertheless, the presence of several serogroups, such as O157:H7, O26, O103, and O111, have already been verified in animals, food, and humans *(9)*. In this sense, more efficient epidemiological monitoring is necessary to control food production, and studies that indicate the incidence of these pathogens can contribute to better assess possible threats and prevent human infections by STEC.

The prevalence rates of STEC in cattle have been determined in several studies; however, it is challenging to compare the results with the various studies published due to differences in sampling and methodologies. The reported STEC prevalence rates range from 0 to 100% in herds *(18)*. During meat processing, these STEC populations transported by healthy livestock can be transferred to carcasses and then to meat. The control of STEC in carcasses and meat products to reduce contamination and minimize the growth of STEC, such as cooling and freezing during the processing and distribution stages of fresh beef, are considered key risk management measures to reduce the presence and risk of STEC in these products *(40)*. The implementation of food safety programs that incorporate hygienic processing and preventive measures for pathogens through the application of good manufacturing practices, sanitation standard operating procedures, and HACCP systems must be adopted in beef slaughterhouses and meat processing establishments. These measures must be taken unconditionally to minimize the risk of contamination of these products, even if the incidence of STEC is very low.

Comparing the negative rates for STEC reported in this study with data from Brazil and other countries, a lower rate than those described in the following is verified: The Department of Agriculture, Livestock and Food Supply of Brazil, through the National Program for the Control of Pathogens, is a food safety system that works to ensure the prevention, shared responsibility, integration, control of the production process, and application of risk analysis to diagnose problems and contribute to obtaining more specific solutions. Data obtained from 2015 to 2020 show an

incidence of less than 0.3% of STEC strains in 6,038 beef and beef trimming samples *(6)*.

FSIS-USDA conducts verification activities for STEC in raw meat products. FSIS considers all nonintact and intact raw meat products as adulterated when it is contaminated with an adulterant, including *E. coli* O157:H7 and these six non-O157 STEC: O26, O45, O103, O111, O121, and O145, when Shiga toxin (*stx*) and intimin (*eae*) genes are present. The reported contamination rate of raw meat samples by STEC has been less than 1% since 2017 *(38)*.

The Rapid Alert System for Food and Feed, a system that reports food safety issues in the European Union, presents data on the contamination of animal or human STEC found in (nonheat-treated) meat products with 261 notifications for the period 2010 to 2020 *(12)*.

The Canadian Food Inspection Agency is an organization that enforces federal food safety regulations for domestic and imported foods in Canada. They presented data related to STEC contamination in raw ground beef, pork, and veal for the period 2013 to 2018, in which they detected three positive samples in 3,273 samples of national origin and no contamination in 48 imported samples *(16)*.

The Australian beef industry reported that the prevalence of O157 STEC and non-O157 STEC strains, respectively, was 0.18 and 0.17% in 136,144 samples of beef trimmings, for the period 2012 to 2017 *(4)*.

The meat companies that supplied the samples analyzed in this study have different production rates and are good sources to investigate the presence of STECs in the Brazilian market. As these meat products exclusively supply the Brazilian local market, it may be important to emphasize that the absence of STEC strains detected in this study showed that these microorganisms seem to be well controlled along the meat chain and, therefore, would not cause diseases for the consumer. Samples of beef cuts evaluated in this study do not present strains from the STEC group, evidenced by the absence of the genes encoding the Shiga toxin (stx_1 and stx_2) in association with the adhesion factor (*eae* gene). The results support the premise that the conditions that these beef cuts samples are processed reduce the possibility of survival and growth of STECs. Finally, the nondetection of STEC in the analyzed samples does not exclude the need for constant tests to monitor the presence of this pathogen in beef produced in local meat chains.

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