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Short communication

Prevalence of molecular markers for *Salmonella* and Shiga toxigenic *Escherichia coli* (STEC) in whole-muscle beef cuts sold at retail markets in Costa Rica

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ABSTRACT

The present study sought to determine the prevalence of molecular markers for *Salmonella* and Shiga toxigenic *Escherichia coli* (STEC) serogroups O26, O45, O103, O111, O121, O145, and O157 in whole-muscle beef cuts sold at retail in urban and semi-rural areas of Costa Rica. A total of 279 (171 urban, 108 semi-rural) samples were purchased from 93 butcher shops between August of 2012 and August of 2013 and tested for the presence of molecular markers characteristic of *Salmonella* and STEC using the DuPont Qualicon BAX® System. The overall prevalence of *Salmonella* and STEC markers was 3.6% (10/279) and 4.7% (13/279), respectively. *Salmonella* markers were more frequently found in semi-rural (6.5%; 7/108) than in urban (1.8%; 3/171) areas. Similarly, STEC markers were more commonly detected in semi-rural (7.4%; 8/108) than in urban (2.9%; 5/171) areas. A marginal association was found between *Salmonella* markers and sampling location ($P = 0.0496$), whereas the presence of STEC markers and sampling location were considered independent ($P = 0.1416$). Among the 13 positive samples for STEC, 38 O-antigen gene fragments were amplified, with serogroups O45 and O121 being the predominant (28.9 and 21.1%, respectively). Markers for somatic antigens O111 and O157 were not detected in any of the samples. The present investigation is the first of its kind in Central America and shows that both *Salmonella* and STEC may be commonly present in whole-muscle raw beef cuts sold at retail markets in Costa Rica. Consequently, consumers may be directly at risk of foodborne illness due if meat products are not adequately cooked and handled in food service or domestic settings. Future work should emphasize continuous monitoring of the presence of these and other zoonotic pathogens in meat and meat products and on the implementation and validation of adequate food safety controls along the farm-to-table continuum.

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1. Introduction

The microbiological safety of foods remains a dynamic situation influenced by multiple factors along the farm-to-table continuum (Newell et al., 2010). Despite great global efforts, the burden of diseases caused by foodborne pathogens remains largely unknown and the information available is limited mostly to industrialized countries and to certain pathogenic microorganisms (Akhtar, Sarker, & Hossain, 2014; Newell et al. 2010). In developing countries, including Costa Rica, multiple information gaps hinder epidemiological investigations and limit the efficacy of public health interventions to reduce the cases of foodborne illness (Käferstein, 2003).

According to data provided by the World Health Organization (WHO) (Boric-Bonifaz, 2008), approximately 70% of acute diarrheal disease in Latin America is thought to be foodborne. The same report indicates that nearly 20% of the foodborne outbreaks that occurred in the region between 1993 and 2002 were attributed to *Salmonella* and that the most common vehicles for the transmission of foodborne pathogens were fish and seafood, water, and red meats, confirmed in 22, 20, and 14% of outbreaks, respectively (Boric-Bonifaz, 2008). More recently, Pires, Vieira, Pérez, Wong, and Hald (2012) developed a probabilistic food attribution model using outbreak data from Latin America and the Caribbean. Based on the summarized data for Costa Rica, 116 outbreaks were reported during 1993 and 2010, of which the majority were attributed to *Shigella* (65), followed by *Salmonella* (20), *Clostridium perfringens*

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(18), pathogenic *Escherichia coli* (7), *Staphylococcus aureus* (5) and *Bacillus cereus* (1), highlighting the relevance of foodborne illness as causes of morbidity in this country (Pires et al., 2012). Eggs, meat products, vegetables, chicken, grains and beans, and pork were the vehicles more commonly associated with the transmission of *Salmonella*, whereas for pathogenic *E. coli*, chicken, pork, dairy, vegetables and water were the sources most frequently linked to human disease in the region (Pires et al., 2012).

The microbiological safety of a variety of food products sold at retail in Costa Rica has been evaluated during the last decade, particularly milk and dairy products (Araya, Davidovich, Chaves, & Arias, 2005; Araya, Gallo, Quesada, Chaves, & Arias, 2008; Arias, Chaves, & Solano, 2010; Chaves & Arias, 2009) and produce (Barrantes & Achí, 2011; Barrantes, Achí, Bolaños, Cerdas, & Cortés, 2012; Calvo et al., 2004; Monge & Arias, 1996; Monge, Chaves, & Arias, 2011; Monge & Chinchilla, 1995). However, the occurrence of pathogens in retail meats has not been studied extensively. Rodríguez, Gamboa, and Vargas (2002) reported on the presence of *C. perfringens* in 36% (28/78) of retail minced meat samples and more recently, Quesada-Gómez et al. (2013) estimated a 2% (4/200) prevalence of toxigenic *Clostridium difficile* in retail meat beef, pork, and poultry purchased in butcher shops in Costa Rica, indicating that this pathogen may pose a potential risk of disease to meat consumers (Quesada-Gómez et al., 2013). However, to date, there is no record in the scientific literature of the presence of other zoonotic pathogens of public health importance in retail raw meats in Costa Rica. In an effort to generate data that contribute to a global understanding of foodborne infections potentially transmitted through beef products, the objective of this study was to determine the prevalence of *Salmonella* and Shiga toxigenic *E. coli* (STEC) serogroups O26, O45, O103, O111, O121, O145, and O157 in whole-muscle beef cuts sold at retail markets in urban and semi-rural areas of Costa Rica.

2. Materials and methods

2.1. Collection of meat samples

A total of 279 samples were collected from 93 retail meat stores (butcher shops or *carnicerías*). Three, whole-muscle beef cuts were purchased per establishment and each facility was visited only once. Samples were collected in urban and semi-rural areas of Costa Rica over a one-year period (August of 2012 to August of 2013) in four visits, evenly separated. Sample collection trips were organized and conducted by the researchers of the International Center for Food Industry Excellence at Texas Tech University (ICFIE-TTU). At the moment of purchase, meat handlers were asked to provide thin beef cuts (1–1.5 cm thick) and to mechanically tenderize the meat (one-pass blade tenderization) prior to packing each beef cut in individual plastic bags. Meat samples were kept cold in thermal bags by means of frozen coolant packs for the duration of the visits to the stores and further processed within 2 h of collection.

2.2. Processing of meat samples

Each beef cut was sponge-swabbed using a sterile sponge pre-hydrated with 10 ml of buffered peptone solution (World Bioproducts; Mundelein, IL, USA). For this purpose, the meat was kept in its original plastic bag as packed by the meat handler and manually extended as much as possible to swab most of its surface on both sides, including the edges. Sponge samples were maintained refrigerated and then transported to the Food Safety Laboratories at ICFIE-TTU in cooler bags with frozen coolant packs via commercial airline within 48 h of meat sample collection. U.S. Department of Agriculture (USDA)'s Animal and Plant Health Inspection Service (APHIS) permits were obtained in advance to take the samples into the United States of America.

2.3. Sample preparation and enrichment

Upon arrival to the laboratory, sponges were manually massaged for 1 min. For *Salmonella* detection, 1 ml of the solution was transferred to 9 ml of 0.1% wt/vol Buffer Peptone Water (BPW; Becton Dickinson, Sparks, MD, USA) and incubated at 37 ± 1 °C for 18 h. For the seven STEC serogroups of interest, 1 ml of the solution was transferred to 9 ml of Tryptic Soy Broth (TSB; Oxoid Ltd., Basingstoke, UK) and incubated at 42 ± 1 °C for 18 h. A USDA-Food Safety and Inspection Service (FSIS)-approved commercial PCR platform was used to detect molecular markers characteristic of *Salmonella* and STEC serogroups O26, O45, O103, O111, O121, O145, and O157 in all sponge samples, using the DuPont Qualicon BAX® System.

2.3.1. PCR detection of Salmonella

Manufacturer's instructions were followed for the standard BAX *Salmonella* PCR assay. Briefly, the lysis reagent was prepared by adding 150 μl of protease to a 12-ml bottle of lysis buffer. After gentle mixing, 200 μl of lysis reagent were placed in cluster tubes and 5 μl of the enriched samples were added to the corresponding tube 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 the lysis step was completed, cluster tubes were inserted into a cooling block chilled at 2–8 °C for at least 5 min. Subsequently, 50 μl of lysate were transferred to clear tubes with optical caps containing all the PCR reagents in a tablet and taken in a chilled blocked to the PCR device. Software instructions were followed and readings obtained after approximately 3.5 h. *S.* Typhimurium ATCC 13311 was used as positive control and ultrapure water was used as blank.

2.3.2. PCR screening of STEC and confirmation of the O antigen

A real-time PCR assay was used to determine the presence of molecular markers for the Shiga toxin (*stx*) and intimin (*eae*) genes in the sponge samples. The methodology is similar to the one described above for *Salmonella*, with the following differences: 20 μl of enriched sample were used to perform lysis and 30 μl of the lysate were used to hydrate the PCR tablets. Software instructions were followed and readings obtained after approximately 60 min. A sample was considered potentially positive for STEC if both *stx* and *eae* gene markers were amplified. All potential positives were subjected to three additional PCR reactions to determine if at least one of the seven O groups of interest was present. For confirmation of the O157 antigen, a standard BAX PCR reaction was conducted under the experimental conditions previously described for *Salmonella* and results were read after approximately 3.5 h. For non-O157 serogroups, two multiplex real-time PCR reactions known as panel 1 (for O26, O111, and O121) and panel 2 (for O45, O103, and O145) were run as described above for the STEC screening. A cocktail of the seven STEC serogroups, all of bovine origin, was used as positive control and ultrapure water was used as blank for all the STEC reactions conducted. All potential positives that amplified for at least one of the O antigens tested were considered presumptive positive (*i.e.*, "positives" for the purpose of this study).

2.4. Statistical analysis

Absolute and relative frequencies of positive *Salmonella* and STEC samples were calculated and 95% confidence intervals for proportions were estimated using the Statistical Analysis System version 9.3 (SAS Institute, Cary, NC). Furthermore, in order to test if

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