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Presence of *Salmonella* and *Escherichia coli* O157 on the hide, and presence of *Salmonella*, *Escherichia coli* O157 and *Campylobacter* in feces from small-ruminant (goat and lamb) samples collected in the United States, Bahamas and Mexico

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ABSTRACT

The objective of this study was to evaluate the frequency of *Salmonella* and *E. coli* O157 found on the hides, as well as presence of *Salmonella*, *Campylobacter* and *E. coli* O157 found in small-ruminant fecal samples from abattoirs and farms in California, New Mexico, Texas, Mexico and the Bahamas. In small-ruminant fecal samples, overall organism presence was identified as 13.9% *Salmonella* (n = 532), 15.3% *E. coli* O157 (n = 477) and 80.7% *Campylobacter* (n = 176). Overall, on small-ruminant hide surfaces, *Salmonella* frequency was 17.1% (N = 339) and *E. coli* O157 was detected at 1.5% (n = 266). The overall lower detection (P < 0.0001) of *E. coli* O157 from hide samples (1.5%) when compared to presence in fecal samples (15.3%), is not consistent with trends expected. Results from this study can be used to better understand and potentially control pathogens in small-ruminants utilized for meat and milk.

1. Introduction

According to the CDC, 2011 estimates, illness acquired from food affect one in six Americans each year, ultimately causing 128,000 hospitalizations and 3000 deaths annually (CDC, 2014). The three major foodborne bacterial targets that have had and continue to have the spotlight for research efforts, surveillance and discussion in the food industry relative to products derived from livestock are *Salmonella*, *Campylobacter* and pathogenic *Escherichia coli* (Newell et al., 2010). Nontyphoidal *Salmonella* and *Campylobacter* are both ranked among the top five pathogens responsible for foodborne deaths, hospitalizations and illnesses (CDC, 2014). Each year in the U.S., an estimated 2138 hospitalizations can be attributed to foodborne acquired *E. coli* O157:H7 (CDC, 2014). These three organisms and their targeted research, however, have focused mainly on cattle or pigs, with less application in small-ruminants. While lamb and goat meat consumption within the U.S. is relatively low compared with beef, pork and poultry, these small-ruminant species provide valuable commodities on a global scale.

Research about frequency of *Salmonella*, *Campylobacter* and *E. coli* O157 associated with small-ruminants is limited, and in need of further investigation to understand the role of these pathogens in ovine and caprine species. An environmental study at an abattoir in Europe found *E. coli* O157 in 2.2%, *Salmonella* in 1.1% and *Campylobacter* in 5.6% of sheep holding pens (Small et al., 2002). While an environmental study does not always indicate pathogens originating from small-ruminants, it presents a risk of contamination on the hide and ultimately carcass of animals processed in that environment. Within the U.S., *E. coli* O157:H7 was identified on 12.8% and *Salmonella* on 14.4% of lamb hides from commercial abattoirs (Kalchayanand et al., 2007). Small abattoirs in Nigeria found *E. coli* O157:H7 on 2.5% of sheep hide swabs and 10% of fecal samples, while goat fecal samples had a prevalence of 5% and no *E. coli* O157:H7 was detected on the hides (Akanbi, Mbah, & Kerry, 2011). In Ireland, *E. coli* O157:H7 prevalence was 5.8% in fleece

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samples but undetected in fecal samples (Lenahan, O'Brien, Kinsella, Sweeney, & Sheridan, 2007). The detection of *E. coli* O157:H7 in these studies from the U.S., Nigeria and Ireland provide some perspective about the role of pathogens in small-ruminants, but each of these studies has some limitation either by limited sampling region or season.

Outbreaks of *E. coli* infection associated with sheep or goats have mostly been linked to raw milk, cheese or petting zoos (Bielaszewska et al., 1997; Caro & Garcia-Armesto, 2007; Espié et al., 2006; Stephan et al., 2008). Research available about the incidence and impact of *Salmonella* contamination in goat meat is especially limited (Duffy, Barlow, Fegan, & Vanderlinde, 2009). Similarly, while some data exists about the prevalence of *Campylobacter* in many other livestock species, there is very limited published research about the prevalence of this organism in sheep and goats (Horrocks, Anderson, Nisbet, & Ricke, 2009).

Considering the diversity of products derived from small-ruminants and their role in the global market, understanding potential pathogens associated with their production is crucial.

The objective of this study was to evaluate the presence of *Salmonella* and *E. coli* O157 found on the hides of lambs and goats, as well as identifying the presence of *Salmonella*, *Campylobacter* and *E. coli* O157 found in small-ruminant feces.

2. Materials and methods

2.1. Sample collection

Hide samples were collected from lambs and goats at small (1–30 animals per day) and large (800–1200 animals per day) sized abattoirs located in California, New Mexico, and Texas over a 14-month period for *Salmonella* (n = 338) and *E. coli* O157 (n = 266) analysis. Each abattoir location visited was sampled over a minimum of 3 days, with no > 30 animals sampled in a single day in order to prevent bias from a single cohort of animals. Hides were swabbed after exsanguination, but prior to hide removal, using a sterile cellulose sponge, pre-moistened with 25 ml of buffered peptone water (BPW, World Bioproducts; Mundelein, Illinois) in an approximately 100 cm^2 area at four locations per each carcass (leg, midline, foreshank, breast/neck) to collect a sample representative of the hide surface. Samples were immediately placed in plastic, insulated coolers kept cold (≤ 4 °C) using previously frozen ice packs. Coolers containing samples were transported to the laboratories at Texas Tech University in Lubbock, Texas, or Angelo State University in San Angelo, Texas for processing.

Small-ruminant fecal samples were obtained from abattoirs located in California, Texas and New Mexico after evisceration by cutting the lower part of the colon, closest to the bung and rectum, with a clean knife and guiding 10–15 g of fecal pellets from the colon into a sterile collection cup. If 10–15 g were not available at the end of the colon, all available sample was collected; no sample from another part of the gastrointestinal tract was collected to make up the difference. Abattoir fecal samples were analyzed for *Salmonella* (n = 286), *E. coli* O157 (n = 270), and *Campylobacter* (n = 21). Farm fecal samples were also collected from sheep and goat farms in the Bahamas, Mexico, California and Texas for *Salmonella* (n = 246), *E. coli* O157 (n = 207), and *Campylobacter* (n = 155). Fresh, sterile gloves were used to pick up farm feces from pens where sheep or goats had been present within 12 h of sample collection, with care being made to collect the most freshly deposited samples possible. Gloves were changed before each new collection was gathered; samples were immediately deposited into a sterile plastic collection cups or whirlpak™ bag (Nasco; Fort Atkinson Wisconsin). A minimum of three animals were in each pen per every one fecal sample collected. Some farms sampled had sheep and goats living together in the same pens, therefore feces collected from the ground were indistinguishable by species. For analysis, these samples were not categorized as either goat or lamb, but instead kept as an independent group designated as “mixed species” and included in the overall analysis for each organism when not differentiated by species.

Fecal samples were immediately placed in plastic, insulated coolers kept cold (≤ 4 °C) using previously frozen ice packs. Coolers containing samples were transported to the laboratories at Texas Tech University in Lubbock, Texas, California State University Fresno in Fresno, California or Angelo State University in San Angelo, Texas for processing. A United States veterinary permit for importation and transportation of controlled materials and organisms and vectors (USDA-APHIS research permit # 114031) was obtained, which granted permission for the transportation of fecal samples from the Bahamas and Mexico to the U.S. for analysis.

2.2. Hide swab sample processing

After arrival to the laboratory, hide swabs in BPW were homogenized for 30 s at 230 rpm using an automated stomacher (Steward Laboratory Systems; Davie, FL) to ensure adequate distribution of microorganisms within the sample bag. *Salmonella* analysis was done using a modification of the USDA FSIS Microbiological Laboratory Guidelines (USDA FSIS MLG, 2014) as described by Gragg et al., 2013. One ml of the sample was added to 9 ml Rappaport-Vassiliadis broth (RV; Oxoid, Hampshire, UK), and 9 ml of tetrathionate broth with iodine (TT; Neogen; Lansing, MI). Enrichments were incubated at 42 °C for 18–24 h. TT and RV enrichments were streaked to Xylose-Lysine-Tergitol agar (XLT4; Hardy diagnostics; Santa Maria, CA) and incubated 18–24 h at 37 °C. Presumptive positive *Salmonella* colonies identified by phenotypical characteristics on XLT4 agar (yellow or red colonies with black centers) were subjected to latex agglutination (Oxoid; Hampshire, UK) for confirmation using manufacturer's instructions. Isolates of colonies which were agglutination positive were selected for further enrichment into Tryptic Soy Broth (TSB; Becton, Dickinson and Company; Franklin Lakes, NJ) for 24 h at 37 °C, and frozen in duplicate with 20% glycerol.

For detection of *E. coli* O157 in the hide samples, 1 ml of sample was added to 9 ml of Gram-Negative broth with vancomycin, cefixime and cefsulodin (GN-VCC; Hardy Diagnostics; Santa Maria, CA) and incubated at 37 °C for 6 h (Echeverry, Loneragan, & Brashears, 2006). Enriched GN-VCC was then subjected to Immunomagnetic Separation techniques (IMS) using anti-*E. coli* O157 Dynabeads® (Invitrogen; Grand Island, New York). IMS product was then plated onto CHROMagar™ O157 with 2.5 mg/l tellurite (CHROMagar; Springfield, NJ) and incubated 24 h at 37 °C. Phenotypically presumptive positive colonies were identified by their mauve color as described with the CHROMagar™ O157 media guidelines (CHROMagar; Springfield, NJ). These presumptive positive colonies were recorded in a lab notebook and confirmed using latex agglutination (DrySpot™ *E. coli* O157 Latex Agglutination Test, Thermo Scientific; Lenexa, KS). Isolates of colonies which were agglutination positive were selected for further enrichment into TSB for 24 h at 37 °C, and frozen in duplicate with 20% glycerol.

2.3. Fecal sample processing

For *Salmonella* analysis, 1 g of each fecal sample was added to 9 ml of TT broth, and 1 g added to 9 ml of RV broth, and incubated at 42 °C for 18–24 h. Procedure for detection, confirmation and freezing of isolates was the same as hide sample processing for *Salmonella*.

For detection of *E. coli* O157, 1 g of fecal sample was added to 9 ml of GN-VCC. The enrichment, culturing, confirmation and freezing of isolates from feces followed the same protocol as hide samples for *E. coli* O157.

For *Campylobacter* analysis, a variation of the USDA FSIS Microbiological Laboratory Guidelines for isolation and identification of *Campylobacter jejuni/coli/lari* from poultry products was used (USDA FSIS MLG, 2014). Five grams of fecal sample was added to a filtered whirlpak™ bag (Nasco; Fort Atkinson Wisconsin) with 30 ml of Bolton broth with selective supplement (EMD Chemicals Inc.; Gibbstown, NJ).