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Salmonella on feces, hides and carcasses in beef slaughter facilities in Venezuela

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ABSTRACT

This study determined *Salmonella* prevalence at different stages during the slaughtering in three beef slaughter plants (A, B and C) located in the western region of Venezuela (Zulia and Lara states). Each facility was visited three times at monthly intervals, from the months October through December of 2006. Samples were collected from hides ($n = 80$), fecal grabs ($n = 80$) and carcasses ($n = 80$) at the phases of pre-evisceration, after-evisceration and pre-cooler at three sampling sites on the animals (rump, flank and brisket). *Salmonella* prevalence was higher on hides (36.3%) than on feces (13.8%) ($P < 0.05$). Differences among slaughter plants for overall *Salmonella* prevalence were observed ($P = 0.001$; A: 3.5%, B: 11.1%, C: 4.4%). From the isolated strains, *Salmonella enterica* subspecies *enterica* ser. Saintpaul, *Salmonella* ser. Javiana and *Salmonella* ser. Weltevreden were identified. Cattle feces and hides might be considered as important sources of *Salmonella* for carcass contamination at different slaughter stages. The presence of potentially pathogenic *Salmonella* serotypes at the slaughtering stages is an evidence of the circulation of this pathogen in the food environment; its presence could increase consumers' risks of infection if proper food handling and preparation techniques are not followed. These data should serve as a baseline for future comparisons in *Salmonella* prevalence on beef carcasses to be used by the government and industry in order to establish preventive measures and to better address the risks of *Salmonella* contamination.

1. Introduction

Developing countries are affected by a wide range of foodborne diseases. The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhea occur every year in developing countries, resulting in 3 million deaths (Alper, 2003). In Latin America and the Caribbean, the Pan-American Institute for Food Protection and Zoonosis (INPPAZ) reported 5283 outbreaks of foodborne disease that affected 174,976 persons and caused 275 deaths between the years 1995 and 2001 (Franco et al., 2003). More recently data collected for Pan-American Health Organization (PAHO/WHO) on developing countries indicated that 9180 foodborne outbreaks were reported from the years 1993 to 2010 from 22 countries of the region, from these outbreaks 69% were caused by bacteria, 9.7% by viruses, 9.5% by marine toxins, 2.5% by chemical contaminants, 1.8% by parasites and 0.5% by vegetal toxins and among bacteria, *Salmonella* spp. was the most frequent agent (Pires et al., 2012), being responsible for 58.1% of the outbreaks and 66.2% of the cases (Franco et al., 2003). Among *Salmonella* serovars circulating in Latin America and the Caribbean, Campos et al. (2012) reported that *Salmonella* ser. Typhi, *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis were the more frequent serovar isolates from human infections in six countries of the region (Argentina, Brazil, Colombia, Costa Rica, Chile and Paraguay). In Venezuela, few studies have been conducted to date on the detection of *Salmonella* spp. in the beef production chain. Nava (2005) screened 15 dual purpose cattle farms ($n = 1463$), recovering *Salmonella* in all of them, with a prevalence that ranged between 1.1% and 55.7%. In beef products, Narváez-Bravo et al. (2005) reported high *Salmonella* prevalence in ingredients (45%) and during beef patty process (up to 66%), and seven *Salmonella* serotypes were reported (Scharzengrund, Braenderup, Sintorf, London, Anatum, Tennessee and Derby). Regrettably, publications addressing the prevalence of *Salmonella* in the beef cattle

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harvest process in Venezuela do not exist according to the authors' knowledge.

Nevertheless, in developed countries, the prevalence of *Salmonella* shedding at feedlots is well documented, as are the dynamics of carcass contamination during the slaughter process, and the specific locations for distribution of pathogens on the carcass (Bell, 1997; Fegan et al., 2005). The ability to consistently identify patterns of contamination on carcasses in processing plants enables the implementation of interventions that target high contamination areas and results in further reduction of pathogens in the beef supply (Rekow et al., 2011). This type of information is lacking in Venezuela, and as a consequence, the establishment of pathogen reduction interventions is unusual at beef processing facilities even though, Venezuelan food regulations establish zero tolerance for *Salmonella* in beef (COVENIN, 1988). Also it is important to mention that the implementation of food safety programs, such as Hazard Analysis and Critical Control Point (HACCP) systems, is not required by law in this country. Therefore, it is important to generate scientific data that will lead to a further understanding of the dynamics of carcass contamination during the slaughter process that will help to develop mitigation strategies for pathogen reduction by the government and industry in order to better address the risks of *Salmonella* contamination.

Furthermore, the objective of this research was to determine the *Salmonella* prevalence in feces, hides and carcasses during the slaughter processes in three distinct slaughter plants located in Venezuela.

2. Material and methods

2.1. Experimental design

This study was carried out in three distinct abattoirs in the western region of Venezuela. The abattoirs were referred to as A, B and C, which kill an average of 900, 300 and 150 animals daily, respectively. The animals slaughtered in these three abattoirs originated in the main beef production regions of Venezuela and represented different breeds (crossbred *Bos indicus* × *Bos taurus*), sex classes (bulls, steers, heifers and cows) and ages (1–11 years). Their diets consisted mainly of grazed material. The slaughter plants were under official inspection to meet quality control, sanitation and hygiene standards during processing.

Each slaughterhouse was sampled three times, at monthly intervals during the months of October, November and December of 2006. At the harvest facility, after each animal was stunned, bled and placed onto the rail system, a sample from each hide ($n = 80$) was obtained. Animals were randomly selected; samples were taken from the following sampling sites: rump, flank and brisket using sponges hydrated with 10 ml of buffered peptone water (BPW) (Difco® Laboratories, Sparks, MD). Approximately a 250 cm^2 area was swabbed at each sampling site. Each of the sampled carcasses was tagged after hide removal, so that the samples were matched from the hide through all of the processing steps.

Carcass sampling ($n = 80$) was performed at three different stages in the slaughtering process of each plant and at three different anatomical sites on the carcasses. The stages in the slaughtering chain were designated as pre-evisceration immediately after de-hiding, after-evisceration immediately after removal of the internal organs and pre-cooler after washing the carcass at the final rail. The sample sites on the carcasses were brisket, flank and rump, as listed in the FSIS's final rule (FSIS, 1996). Beef carcass samples were collected using sponges hydrated with 10 ml of BPW. During collection of the samples, care was taken to avoid cross contamination. On each of the carcass's sample sites, an area of 100 cm^2 was swabbed using the sponge technique and disposable sterile templates.

Intestinal feces samples were collected after evisceration from each tagged carcass ($n = 80$). The entire gastrointestinal tract was tagged and followed to the viscera room. Once there, the recto-colon portion of the intestine was cut and put individually into a labeled sterile bag.

All samples were transported in coolers containing ice packs, and were received and processed in the microbiology laboratory in the Veterinary Science Building at Zulia State University within 24 h of collection.

2.2. Salmonella detection

For isolation of *Salmonella* from hide and carcass samples, 10 ml of BPW was added to each sponge bag, for a total volume of 20 ml. Each of the sponges was homogenized, by hand massage for 2 min, then 1 ml of homogenized sample was added to each 9 ml of Rappaport Vassiliadis broth (RV), (Himedia®) and Tetrathionate broth (TT) (Difco®).

For fecal samples, sterile scissors were used to open the colon–rectum samples. Samples were processed according to methods previously described by Dargatz et al. (2000) and Narváez-Bravo et al. (2013). Briefly, 1 g of feces was weighed and added to each of the 9 ml RV and TT broths. After the addition of the samples to the enrichment broth, each tube was homogenized and incubated at 42 ± 0.5 °C for 24 h. After incubation, TT and RV enrichment for hides, carcasses and fecal samples were streaked onto XLT4 (Difco®) and Hecktoen Enteric (Himedia®) agar medium and incubated for 24 h at 37 ± 0.5 °C. Negative plates were incubated for an additional 24 h at 35 °C. All presumptive colonies (at least five characteristic colonies were tested for each media type plate, if available) were screened through the following biochemical tests: triple sugar iron (TSI) and LIA slants. All presumptive *Salmonella*, based on TSI and LIA outcome, were subjected to additional biochemical tests: urea, Voges–Proskauer (VP), methyl red (MR), indole, citrate, potassium cyanide, malonate, dulcitol, lysine, ornithine and arginine. All of the isolates with typical results for *Salmonella*, on the biochemical tests mentioned above, were tested for somatic antigens using polyvalent O antiserum (Difco®), following the manufacture recommendations. Some of the isolates were sent for serotyping at the Bacteriology Laboratory at the Medical School, Zulia State University. Once the purity of the submitted isolated material was tested, the strains underwent a complete set of biochemical tests (indole production, methyl red, Voges–Proskauer, Simmons Citrate, hydrogen sulfide on TSI, urea hydrolysis, phenylalanine deaminase, lysine decarboxylase, ornithine decarboxylase, arginine dehydrolase, motility, gelatin hydrolysis, grown in KCN, malonate utilization, D-glucose-acid, D-glucose-gas, fermentation of lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, myo-inositol, D-sorbitol, L-arabinosa, raffinose, L-rhamnose, maltose, D-mannose, D-xylose, threalose, D-arabitol, glycerol, cellobiosa, mellibiose, esculin hydrolysis, acetate utilization, DNase, nitrate–nitrite and oxidase) and polyvalent somatic antisera. Once the confirmation step for generic *Salmonella* was completed, the serotypes were designated according to the Kauffmann–White scheme (Grimont and Weill, 2007) using Denka Seiken (Tokyo, Japan) Agglutinating antisera (somatic and flagellar) following manufacturer recommendations.

Reference *Salmonella* strains (ATCC 123215 and 9842) were used as positive controls to evaluate the quality of the media and reagents used in this research.

2.3. Statistical analysis

The data collected was analyzed using SAS (Cary, NC) version 9.2 (SAS, 2003). For each pathogen, a Chi-squared analysis (Fisher's exact test) was used, to test for differences among plants, slaughter processes and anatomical sites.

3. Results and discussion

3.1. Salmonella detection on hide and fecal samples

The overall prevalence of *Salmonella* on hides and intestinal feces is shown in Table 1. Positive samples for *Salmonella* were greater on hides than on feces ($P = 0.001$, 36.7% vs. 13.8%; respectively). However,