



**Frequency of contamination and serovars of *Salmonella enterica* and *Escherichia coli* in an integrated cattle slaughtering and deboning operation**



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**Abstract:**

This study aimed to determine the frequency of contamination and serovar diversity of *Salmonella enterica* (SE) and *Escherichia coli* (EC) in different stages of cattle slaughtering and deboning processes. Fecal, carcass, and primal cut (100 of each type) samples were collected in a Federally Inspected slaughterhouse in Mexicali, Baja California. EC was not analyzed in fecal samples because it is part of the gut microbiota. Strain identity was confirmed by biochemical methods and PCR, using the taxonomic genes *invA* and *gadA* for SE and EC, respectively. In EC, the presence of genes associated with the main pathotypes was also investigated. SE had a 34 % frequency in fecal samples, 3% in carcasses, and 2% in cuts, while Montevideo was the predominant serovar (72.5 % of the total strains). EC was detected in carcasses (34 %) and cuts (11 %) at an average concentration of 0.012 and 0.33 log CFU cm<sup>-2</sup>, respectively. Although several of the identified EC serovars were associated with enterotoxigenic or Shiga toxin-producing strains, none carried the virulence factors typically observed in these pathotypes. In summary, beef carcasses and cuts are not a relevant source of EC pathogenic strains. However, beef is an important reservoir of SE, which represents a public health risk. Genomic studies are required on the virulence profile and genes of SE strains commonly associated with subclinical infections and isolated from apparently healthy animals.

**Key words:** *Escherichia coli*, *Salmonella* spp., Cattle, Slaughter, Serovars, Pathotypes.

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## Introduction

Intestinal infections caused by *Salmonella enterica* and the different pathotypes of *E. coli*, such as the Shiga toxin-producing *E. coli* (STEC), constitute a global public health problem<sup>(1)</sup>. Both pathogens are common contaminants of meat from different species, including beef<sup>(2,3)</sup>, which is the second most widely consumed meat type in Mexico<sup>(4)</sup>. Therefore, the characterization of circulating strains of *S. enterica* and *E. coli* in the beef cattle production chain is crucial to improve management of the risks associated with both pathogens.

In Mexico, most of the studies in this field focus on a single point in the production chain. For example, several authors have observed moderate frequencies (8 to 15 %) of *Salmonella* spp. in beef carcasses<sup>(5-7)</sup>, although the represented serovars are not reported in all cases.

More comprehensive studies report higher levels of contamination (25 to 100 %) in hides, feces, lymph nodes, non-refrigerated carcasses, and meat samples<sup>(8-10)</sup>, as well as the predominance of certain serovars in some of the matrices analyzed. However, the comparison between studies is difficult due to variations in sample type, step of the production chain, method of analysis, geographical location, animal production system, and sanitary conditions of the studied process.

Regarding *E. coli*, the situation is similar. Most of the studies focus on a specific fragment of the production chain and deal with enterohemorrhagic STEC strains, such as *E. coli* O157:H7<sup>(6,11,12)</sup>. Although previous studies have reported a low frequency (1 to 3%) of pathogenic *E. coli* strains in bovine carcasses and feces<sup>(11-13)</sup>, their distribution has not been thoroughly explored throughout the production chain. This information can contribute to identifying dissemination patterns in different processes and geographic regions, as well as measures to guarantee food safety and protect public health. Therefore, this study aimed to determine the frequency of contamination and serovar diversity of *S. enterica* and *E. coli* in a Federally Inspected slaughterhouse with horizontal integration of cattle slaughtering and deboning processes.

## Material and methods

### Study design and sample size determination

Samples were collected in three stages of the beef transformation process, from slaughtering to deboning: 1) Rectal contents collected after evisceration, 2) Hot carcasses, and 3) Primal cuts. Each stage was considered as an independent sampling, since it was not possible to determine in advance the destination of the animals, which were sold either as whole carcasses or as primal cuts. The sample size for each evaluated stage was calculated with the statistical equation used to determine the sample size of a population proportion when the number of elements in that population is unknown<sup>(14)</sup>:

$$n = \frac{Z_{\alpha}^2 * p * q}{d^2}$$
 ; n=sample size;  $Z_{\alpha}^2 = Z$  value in a normal distribution  $Z_{\alpha} = 1.96$  when  $\alpha = 0.05$ ; p= population proportion with the studied characteristic (if unknown, 0.5 is used, as in this case); q= population proportion without the studied characteristic (1-p); d= desired error or precision, fixed at 10% (0.1).

With this formula it was obtained a sample size per stage of 96, which was rounded to 100, for a total of 300 samples in the study. The study was performed in September 2013 in an integrated beef production company in Mexicali, Baja California, comprising feedlots, slaughtering, and deboning operations. The sampled carcasses belonged to crossbred *Bos indicus* young bulls, with an average age of 24 to 30 months, originating from eight Mexican states and finished during an average of 190 days in the feedlot. The company was selected

due to its level of integration, which allows having a production chain model in a single place. The slaughterhouse is 1 km off the feedlots and can process 300 heads of cattle per 8-h shift.

## **Sampling**

### **Rectal contents**

Fecal samples were collected from the rectum after evisceration, at 20 min *postmortem*. Approximately 100 g of fecal material were collected from each rectum. For this, viscera packages were momentarily held in the evisceration ramp. The rectum ligature was cut open and, using new nitrile gloves, it was collected the fecal samples in sterile bags, which were kept inside insulated containers with refrigerated gels ( $\approx 4$  °C) until further processing in the laboratory. It was used a new pair of gloves for each sample. As *E. coli* is part of the gut microbiota of cattle, it was assumed that all fecal samples will test positive and have high concentrations of EC. Therefore, fecal sampling was performed only for *S. enterica*, not for EC.

### **Hot carcass sampling**

Carcass sampling was conducted according to the methodology employed by the United States Department of Agriculture for the microbiological baseline studies for cattle<sup>(15)</sup> with slight modifications. Instead of refrigerated carcasses, were sampled hot carcasses, and was used the peptone water from the same hot carcass to detect *E. coli* and *S. enterica*. Carcass swabs were collected from three different areas (leg, skirt, and brisket) of right halves. For that purpose, were used sponges pre-moistened with 10 ml of buffered peptone water and 10 x 10 cm<sup>2</sup> sterile disposable frames (Meat/Turkey Carcass Sampling Kit, NASCO®, USA). The total sampling area per carcass was 300 cm<sup>2</sup>.

### **Cuts**

Once the primal cuts, were obtained in the cutting room and before packaging, legs, skirts, and briskets were randomly selected for sampling. It was followed the same method previously described for carcasses, except that it was used a single 100 cm<sup>2</sup> frame per cut.

## **Microbiological analysis**

Once the samples were taken, sponges were sealed in sterile plastic bags and kept inside insulated containers with refrigerant gels ( $\approx 4$  °C) for transfer to the plant laboratory. It was inoculated in triplicate 100 µl of the peptone water samples in Salmonella-Shigella (SS) agar plates (MCD Lab®, PRONADISA-CONDA®, Spain). Plates were incubated at 37 °C and

examined for growth at 24, 48, and 72 h. Plates without *Salmonella* spp. characteristic growth at 72 h were considered negative. For the fecal samples, was used sterile swabs for direct streaking in SS medium and followed the same procedure for plate incubation and reading. Colonies with typical *Salmonella* spp. morphology (round, convex, regular border colonies, with hydrogen sulfide production) were restreaked in CHROMAgar Salmonella Plus medium (CHROMAgar®, France) for purification and identification. All colonies suggestive of *Salmonella* spp. (hydrogen sulfide producers or purple in CHROMAgar Salmonella Plus) were recovered. The pure and confirmed isolates of *Salmonella* spp. in selective and differential media were streaked in trypticase soy agar (TSA, MCD Lab®, PRONADISA-CONDA®, Spain) for their identification through biochemical methods and PCR. From the remaining volume of peptone water, it was took 1 ml for each rehydratable 3M Petrifilm® *E. coli*/Coliforms (3M, USA) to estimate the concentration of generic *Escherichia coli*. Following the instructions provided by the manufacturer, 3M Petrifilm plates were incubated at 37 °C and analyzed at 24 and 48 h. It was used the CHROMAgar ECC medium (CHROMAgar®, France) to isolate the strains identified in the 3M Petrifilm plates.

### **Biochemical identification**

*Salmonella* strains were identified with substrates prepared in the laboratory according to the results of the following tests<sup>(16)</sup>: triple sugar iron (TSI); hydrogen sulfide, indole, and motility (SIM); Simmons citrate; urea; methyl red and Voges-Proskauer; malonate-phenylalanine; gluconate; arginine, ornithine, lysine, and control decarboxylase enzymes. *Salmonella enterica* subsp. *enterica* ser. Typhimurium ST19 was used as a positive control. This strain was obtained from the culture collection of the Hospital General Dr. Manuel Gea González, in Mexico City, isolated and characterized by VITEK 2 (bioMerieux, France)<sup>(17)</sup>. The same tests were used for *E. coli*, except for amino acid decarboxylation<sup>(16)</sup>, using a strain of *E. coli* K12 was used as a positive control.

### **Molecular identification**

Molecular identification was carried out by end-point PCR, using specific gene sequence primers typical of each species (Table 1). Genomic DNA was extracted from the purified strains previously refreshed in tryptic soy broth (MCD Lab®, PRONADISA-CONDA®, Spain) for 18 to 24 h using the “DNeasy Blood & Tissue Kit” (Qiagen, Inc., USA), following the instructions provided by the manufacturer. For *S. enterica*, was used the *invA* gene<sup>(18)</sup>, and for *E. coli*, the *gadA* gene<sup>(19)</sup>, which codes for the alpha subunit of the glutamate decarboxylase. Additionally, to identify the different pathotypes, was included six genes associated with enteropathogenic (EPEC), enterotoxigenic (ETEC), and Shiga toxin-producing (STEC) strains. Among these, the *eaeA* gene codes for an intimin, an important protein for adhesion through the translocated intimin receptor<sup>(20)</sup>. This gene is present in the

genome of EPEC and STEC pathotypes. Moreover, the genes coding for Shiga toxins 1 (*stx1*) and 2 (*stx2*) usually occur in STEC strains, which show the same phenotype when they carry one or both of these genes<sup>(20)</sup>. It was also studied the presence of genes coding for the heat-stable (*estA*) and heat-labile (*eltA*) toxins associated with ETEC<sup>(21)</sup> strains; as well as the *bfp* gene (*bundle forming pilus*), involved in adhesion to the intestinal epithelium, which is found in the genome of EPEC strains<sup>(2)</sup>. The PCR reactions were carried out in a total volume of 25 µl and the reagents from the Top Taq Master Mix Kit (QIAGEN®, USA) were used with the following final concentrations: 1.25 Units of Taq Polymerase, 1.5 mM of MgCl<sub>2</sub>, 1x PCR Buffer, 200 µM of each dNTP. The conditions used for each reaction were as described in previous publications (Table 1).

**Table 1:** Genes and primers used in the molecular characterization of *Salmonella* spp. and *Escherichia coli*

Pathogen	Gene	Amplified fragment (bp)	5'→3' Primer sequence	Ref.
<i>Salmonella</i> spp.	<i>invA</i>	284	139 GTGAAATTATCGCCACGTTTCGGGCAA 141 TCATCGCACCGTCAAAGGAACC	(19)
	<i>gadA</i>	670	<i>gadA</i> 1: ACCTGCGTTGCGTAAATA <i>gadA</i> 2: GGGCGGGAGAAGTTGATG	(20)
	<i>eaeA</i>	890	EAE1: GTGGCGAATACTGGCGAGACT EAE2: CCCCATTCCTTTTTACCGTCG	(21)
	<i>stx1</i>	582	STX1F: ACACTGGATGATCTCAGTGG STX1R: CTGAATCCCCCTCCATTATG	(21)
<i>E. coli</i>	<i>stx2</i>	255	STX2F: GGCAGTGTCTGAAACTGCTCC STX2R: TCGCCAGTTATCTGACATTCTG	(21)
	<i>estA</i>	190	STa-F CTAATGTTGGCAATTTTTATTCTGTA STa-R AGGATTACAACAAAGTTCACAGCAGTAA	(22)
	<i>eltA</i>	132	LT-1 AGCAGGTTTCCACCGGATCACCA LT-2 GTGCTCAGATTCTGGGTCTC	(22)
	<i>bfp</i>	324	EP1, CAATGGTGCTTGCGCTTGCT EP2, GCCGCTTTATCCAACCTGGT	(2)

Amplified PCR products with high (*gadA*, *eaeA*, *stx1*) and low molecular weights (*eltA* and *estA*) were subjected to a 1% and 2% agarose gel electrophoresis (SeaKem® LE Agarose, Lonza, ME, USA), respectively. Gels were run in a tris/borate/EDTA buffer (TBE 1x) at 80 V for 50 min using SYBR Safe DNA Gel Stain (Invitrogen, USA) to reveal the DNA fragments. The visualization and digitization of images were performed in a Gel Logic 2200 imaging system (Kodak, USA) with the Care Stream® software (Carestream Health, Inc., USA). The same strains of both pathogens referred to in the biochemical identification were used as positive controls. Furthermore, to identify the *E. coli* pathotypes, we included strains of EPEC, ETEC, and STEC as controls. These strains were also obtained from the culture

collection of the Hospital General Dr. Manuel Gea González and were previously characterized by VITEK 2.

## Serotyping

### *Salmonella* spp.

**Serotyping of the somatic antigen (O).** The serological identification of *Salmonella* strains was performed using the Kauffmann–White scheme<sup>(22,23)</sup>. The somatic antigen (O) was obtained by boiling the bacterial cultures ( $\approx 94$  °C) for 1 h. The O antigen was determined using polyvalent anti-O sera A, B, D, D, E, F, and G (DIFCO, BD) and monovalent (specific) anti-O sera from serogroups A, C, D, E, and F (DIFCO, BD).

**Serotyping of the flagellar antigen (H).** This antigen was obtained by inoculating the strains in a semisolid medium in Cragie's tubes and subculturing them in nutrient broth. Phase I and II H antigen were determined using the H antiserum Spicer-Edwards system (DIFCO) and monovalent sera (specific) from serogroups A, B, C, D, E, and F.

Although serovar determination was not carried out in a reference laboratory, the complete genome of the obtained strains was sequenced as part of another investigation<sup>(24)</sup>. This allowed to confirm, through *in silico* raw sequence analysis, the preliminary serotyping results and to determine the serovar of strains that were untypeable by biochemical methods.

### *E. coli*

*E. coli* strains were serotyped by microagglutination in a 96-well microplate using 187 somatic antigen (O) antisera, and 53 flagellar antigen (H) antisera from rabbit (SERUNAM), following the method described by Ørskov and Ørskov<sup>(25)</sup>, with minor modifications.

**Phylogroup classification.** As certain *E. coli* phylogroups are associated with animals or humans, as well as with different bacterial pathotypes, it was decided to perform the classification into phylogenetic groups by PCR, according to the Clermont scheme<sup>(26)</sup>. This technique allows to divide the *E. coli* isolates into seven species-characteristic phylogenetic groups (A, B1, B2, C, D, E, and F) and one additional group, which corresponds to Cryptic Clade I. The test was performed by a quadruple PCR to detect the *arpA*, *chuA*, *yjaA*, and

*TSPE4.C2* genes. Moreover, when results suggest phylogroups E and C, an additional duplex PCR is performed for an allelic variant of the *arpA* (specific for group E) or *trpA* gene (specific for group C), including an internal control directed to the *trpBA* gene. Reactions were performed directly from fresh colonies grown for 24 h in TSA agar. The PCR reactions were performed in a 25 µl volume, under the same conditions previously described<sup>(26)</sup>. The PCR amplification products were subjected to a 2% agarose gel electrophoresis (SeaKem® LE Agarose, Lonza, USA) at 80 V for 50 min. Visualization and digitization of images were performed as previously described for *S. enterica*. *E. coli* K12 and representative strains of each phylogroup were included as positive controls. These strains were obtained from the culture collection of the Hospital General Dr. Manuel Gea González, previously classified according to the Clermont scheme<sup>(26)</sup>.

### Data analysis

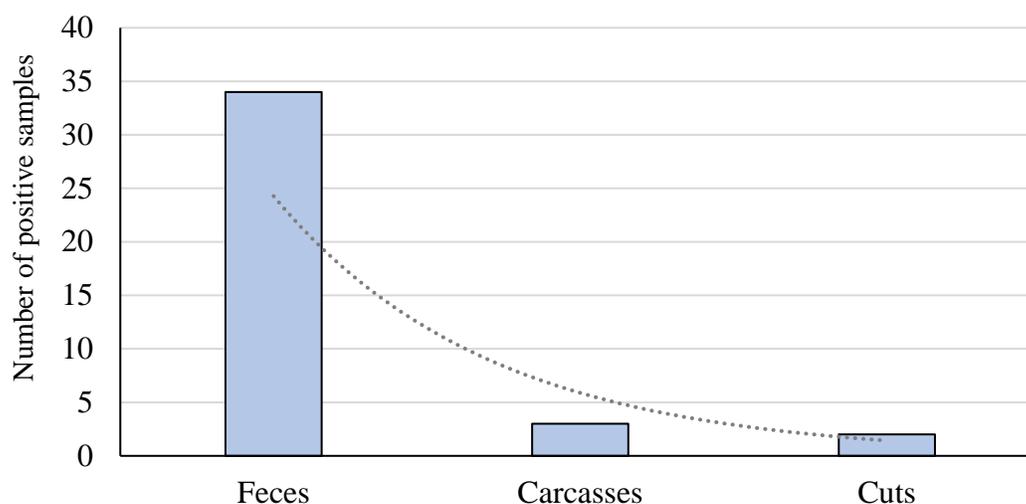
It was calculated the frequency of contamination for both pathogens in the evaluated samples. Concentration was only determined for *E. coli*. The Chi-square test and the odds ratio were used to test if there was association between pathogen positivity and sample type.

## Results

From the analyzed samples (300 for *Salmonella* spp. and 200 for *E. coli*), it was obtained 84 isolates. Of these, 39 were identified as *Salmonella* spp. and 45 as *E. coli*, with a global frequency of 13.0 and 22.5 %, respectively. Only one carcass sample was positive for both bacteria.

### *Salmonella* spp.

The frequency of contamination with *Salmonella* spp. was 34 % in fecal samples, and 3 and 2 % in carcasses and cuts, respectively (Figure 1). All these isolates were identified as *Salmonella* spp. by biochemical assays and PCR. Initially, it was identified two additional strains with positive results based on the biochemical tests and PCR, but they were untypeable by biochemical methods. However, when confirming the serovar by *in silico* raw sequence analysis, these two strains were identified as *Pseudomonas putida*, a species also carrying the *invA* gene<sup>(27)</sup>, and were therefore discarded.

**Figure 1:** *Salmonella* spp. in bovine feces, carcasses, and cuts (n=100 for sample type)

The Chi-square test evidenced a strong association ( $\chi^2=58.5$ ,  $P<0.0001$ ) between sample type and frequency of contamination with *Salmonella* spp. (Table 2). This was confirmed by the odds ratio, according to which the probability of finding positive samples for *Salmonella* spp. in feces was 20.1 times higher compared to the other matrices.

**Table 2:** Association between the frequency of contamination with *Salmonella* spp. and *Escherichia coli* and sample type

Sample type	n	Positivity %	Odds ratio	95% C.I. <sup>1</sup>	$\chi^2$	$P^2$
<i>Salmonella</i> spp.						
Feces	100	34	20.1	7.5-53.5	58.5	<0.0001
Carcasses/cuts	200	5				
<i>E. coli</i>						
Carcasses	100	34	4.2	2.0-8.8	15.2	<0.0001
Cuts	100	11				

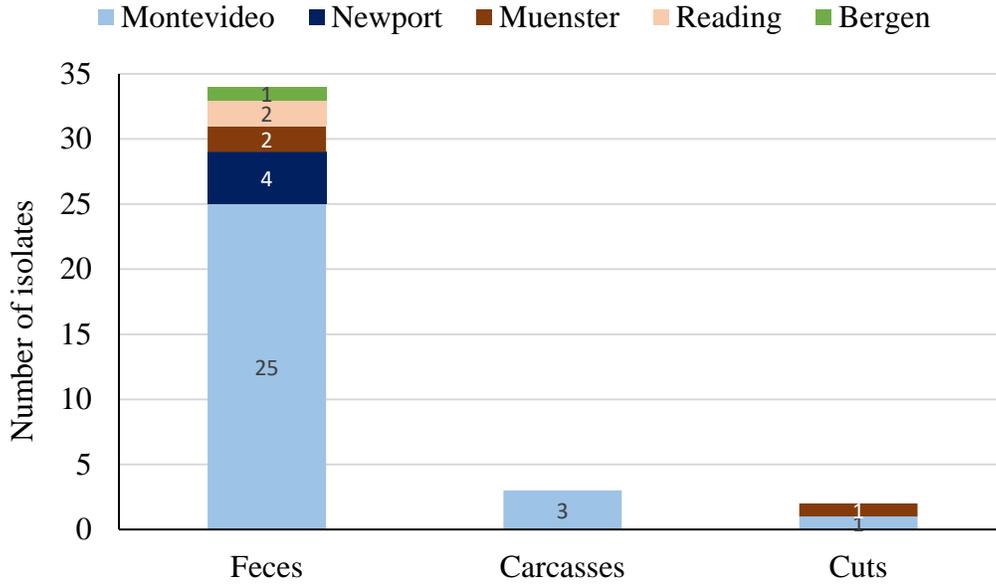
<sup>1</sup>95% confidence interval for the odds ratio.

<sup>2</sup>Significance level (probability)

Regarding the serovars (Figure 2), it was possible to typify 35 of the 39 isolates by serological methods. The remaining four strains were only partially characterized. Since they had a rough O antigen, it was only possible to obtain a partial antigenic formula based on the flagellar antigen. However, the *in silico* analysis, with raw reads from the fully sequenced genomes reported in another study<sup>(24)</sup>, allowed determining the serovar of 100% of the isolates. In total, it was identified five serovars: Bergen (n= 1), Reading (n= 2), Muenster (n= 3), Newport (n= 4), and Montevideo (n= 29). All Montevideo isolates were monophasic for the H antigen, although the antigenic formula allowed the identification of two subgroups within this serovar, 22 of them coming from feces, carcasses, and cuts, with the formula

6,7:g,m,s: -, while the seven remaining strains, all from fecal samples, had the formula 6,7:g,m,p,s:-.

**Figure 2:** Distribution of *Salmonella enterica* subsp. *enterica* serovars according to isolation source (n=100 per sample type)



The serovar distribution per sample type showed *Salmonella enterica* subsp. *enterica* ser. Montevideo was present in all the analyzed matrices. Conversely, strains of *Salmonella enterica* subsp. *enterica* ser. Newport and Reading, detected at a lower frequency than *Salmonella* Montevideo, were only detected in fecal samples.

### *E. coli*

*E. coli* was detected in 34 % of the carcasses and 11% of cuts. A strong association ( $\chi^2=15.2$ ,  $P<0.0001$ ) was evidenced between sample type and frequency of contamination with *E. coli* (Table 2). This was confirmed by the odds ratio, which demonstrated that the probability of finding positive samples in carcasses was higher than in cuts (odds ratio: 4.2, 95% confidence interval: 2.0-8.8,  $P<0.0001$ ).

In cuts, the positive samples were distributed in a relatively uniform way, with five strains from isolated from the brisket, three from the skirt, and three from the leg. The concentration of this bacterium was low, both in carcasses and cuts, with values between 1 and 8 CFU  $\text{cm}^{-2}$ . Of the 45 isolated and identified strains using 3M Petrifilm plates and CHROMAgar ECC, 41 showed a phenotype characteristic of the species. The four remaining strains, isolated from carcasses, showed atypical results; three were indole negative and slow lactose-

fermenting, and one was positive for citrate, malonate, and cellobiose. However, all strains were molecularly confirmed by PCR, using the *gadA* gene as a taxonomic marker.

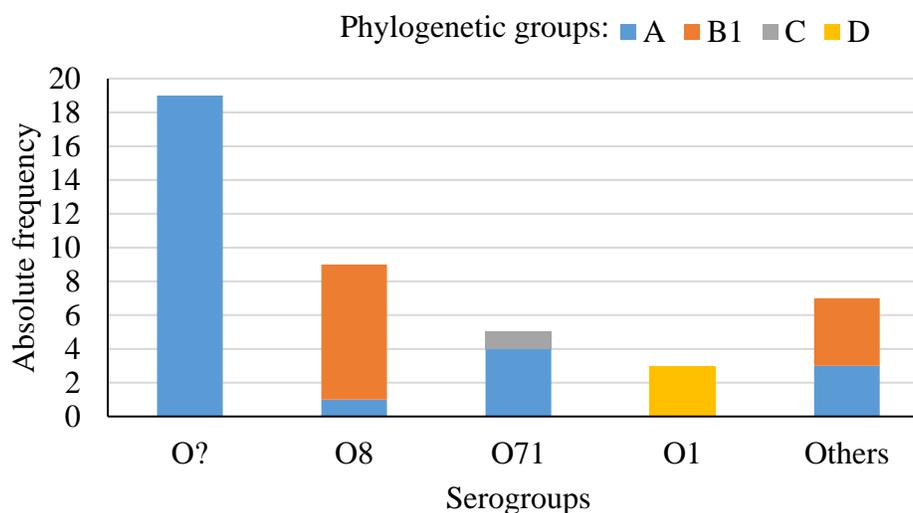
A total of 31 *E. coli* serovars were totally or partially identified (Table 3). The most frequent serogroups were O8 (29%) and O71 (19.4%), and the most common serovar was O1:H6 (9.7%).

**Table 3:** Frequency of *Escherichia coli* partially or totally identified serovars by sample type

Sample type	n	Serovar
Carcass	1	O28ab:-
	3	:-H30
	1	:-H32
	3	O1:H6
	1	O113:-
	1	O154:H21
	1	O156:-
	1	O166:H21
	1	O32:-
	1	O6:-
	5	O8:-
	1	O8:H19
	1	O8:H2
	1	O8:H21
Leg	1	:-H32
Brisket	1	O124:-
	1	O71:-
	2	O71:H12
	1	O8:H8
Skirt	1	O7:H39
	2	O71:H12

The predominant phylogenetic groups were A (60 %) and B1 (26.7 %), group B2 was absent, and groups C and D occurred at low frequencies (2.2 and 6.7 %). There were two strains with inconclusive results; therefore, they were not assigned to a phylogroup. It was interesting to observe how some serogroups were strongly associated with certain phylogenetic groups. In the serogroup O8, 8 out of 9 strains belonged to phylogroup B1. Similarly, 4 out of 5 strains in the serogroup O71 belonged to phylogroup A, and all strains from serogroup O1 belonged to phylogroup D (Figure 3).

**Figure 3:** *E. coli* phylogenetic groups, based on the Clermont scheme<sup>(26)</sup>, represented in each of the identified serogroups (n=43)



## Discussion

In several developed countries, with intensive beef production systems similar to those in developing countries, like Mexico, the contamination frequency of *Salmonella* spp. tends to be low in carcasses, meat, and feces<sup>(28-30)</sup>. However, in this study, it was observed a moderately high contamination frequency in feces, which coincides with previous reports in other Federally Inspected slaughterhouses in the country<sup>(9)</sup>. This indicates that, in Mexico, beef cattle farms may constitute an important reservoir of this pathogen. This surely represents an important challenge for the interventions applied during slaughter. Although the frequency of contamination in carcasses is drastically reduced compared to feces, a total control of the pathogen is not achieved. Furthermore, *Salmonella* was also detected in primal cuts, which shows the dissemination potential of this pathogen along the production chain. This is demonstrated by the detection of strains of the same serovar in feces, carcasses, and cuts. Additionally, these results are similar to those of previous studies (2 to 30 % positivity to *Salmonella*) in meat samples in supermarkets<sup>(31,32)</sup>, which only sell meat from Federally Inspected slaughterhouses. This situation implies a more complicated epidemiological situation in the commercialization chains associated with municipal slaughterhouses, which lack the infrastructure and sanitary conditions of those under federal inspection<sup>(33)</sup>. In fact, the positivity frequency to *Salmonella* in retail beef samples originated from municipal slaughterhouses generally exceeds 50 %<sup>(10,34)</sup>.

The above analysis shows the need to reinforce the control measures for *Salmonella* spp. in live animals, since the interventions applied on farms are limited. Therefore, the evaluation of the prevalence of *Salmonella* spp. in calves entering feedlots, the monitoring of infected animals and their separate management, and thus the detection of possible reservoirs are just some of the measures that could help to reduce the percentage of *Salmonella* carrier animals in slaughterhouses.

All the isolated serovars have been previously associated with human infections in Mexico<sup>(35)</sup>; therefore, the risk pose by these strains to public health should not be minimized. The clear predominance of *Salmonella* Montevideo in the evaluated processes is notorious and surprising, considering that the participating company fattens animals from eight Mexican states. The absence of previously common serovars in samples from Mexican beef cattle, such as Typhimurium, Anatum, and Agona, is also interesting<sup>(36)</sup>. Although previous studies have reported a variable distribution of *Salmonella* spp. serovars through time and between geographical areas and studies, the predominance of *Salmonella* Montevideo in this study is consistent with the increasing prevalence of this serovar in North America<sup>(37,38)</sup>. Furthermore, recent studies conducted in Mexico reported Montevideo and Reading serovars, but not Typhimurium, in strains isolated from cattle feces, carcasses, and lymph nodes<sup>(8,9)</sup>. In any case, it is difficult to determine the factors associated with the prevalence of specific strains in animal production systems without resorting to molecular studies to evaluate the genetic diversity of populations and the presence of genes associated with virulence, environmental persistence, and subclinical infections. However, these results indicate that apparently healthy cattle can carry *Salmonella* spp. at moderately high frequencies and that this pathogen can spread beyond the slaughtering process, with consequent risks to food safety.

The frequency of contamination with *E. coli* was similar to that of *Salmonella* in carcasses, and higher in cut samples; however, this bacterium occurred in low concentrations (<8 UFC cm<sup>-2</sup>). Although *E. coli* is part of the normal intestinal microbiota, the interventions applied in the slaughterhouse reduced three times the frequency of this bacterium in cut samples, in which the probability of finding positive samples was lower than in carcasses. Furthermore, in Mexico, the circulation of pathogenic strains in cattle appears to be lower than in other countries, such as the United States of America, where they are considered a public health problem<sup>(39)</sup>. This was further confirmed by the absence of the virulence factors associated with the STEC, EPEC, and ETEC pathotypes in the studied samples. Moreover, these findings coincide with previous studies<sup>(13)</sup> that reported serovars (O157 and not-O157) associated with STEC strains (n=146), but only two of these carried the characteristic virulence factors. In Mexico, subsequent studies showed the same trend, reporting low rates (<1%) of contamination with pathogenic strains of *E. coli* in carcasses and ground beef<sup>(9,40,41)</sup>. This behavior could derive from multiple factors. Among these, the photoperiod, longer during the summer in the northernmost countries, has been considered responsible for the

marked seasonal effect on the prevalence of pathogenic *E. coli* in cattle. Other authors have suggested that the circulation of different enterobacteria, with cross-reaction of somatic (O) antigens, could be a negative selection factor of *E. coli* pathotypes in Mexican cattle populations<sup>(42)</sup>. This is in line with the high percentage of serum samples, from apparently healthy cattle, with a bactericide response against *E. coli* O157 (71 %), in herds from central Mexico<sup>(43)</sup>.

Regarding the identified *E. coli* phylogroups, the predominance of A and B1 is similar to what is commonly observed in strains of animal origin<sup>(44,45)</sup>. In line with the absence of virulence genes associated with pathotypes, only one strain was classified in group C, to which other STEC strains of animal origin belong<sup>(44,46)</sup>. However, practically all the identified serovars have been associated with the STEC or ETEC pathotypes, which are important in foodborne diseases<sup>(47-49)</sup>. The potential health risks of non-pathogenic strains should not be overlooked since they could acquire virulence factors by incorporating plasmids or phages<sup>(50,51)</sup>. Hence, further research is needed in this area.

## **Conclusions and implications**

This study shows that nearly one third of the cattle approved for slaughter carry different serovars of *Salmonella enterica* in their feces, despite being apparently healthy animals. Furthermore, the results show the ability of the pathogen to spread to the following segments of the production chain, with the consequent risks to public health. Hence, it is important to conduct further studies on the genetic factors of *S. enterica* associated with the establishment of subclinical infections in cattle and their persistence in livestock populations. Moreover, the results for *E. coli* show, as in other regions of the country, a low circulation of pathogenic strains of *E. coli* in beef carcasses and cuts. However, the analyzed samples were obtained from a single slaughterhouse, and the scope of this study, for *E. coli*, does not consider hide or feces samples, in which the probability of finding pathogenic strains is higher.

## **Acknowledgments and conflicts of interest**

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