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Original Article

Characterization and Molecular Subtyping of Shiga Toxin–Producing *Escherichia coli* Strains in Butcher Shops

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Abstract

Shiga toxin–producing *Escherichia coli* (STEC) are important emerging foodborne human pathogens. Ruminants are the main animal reservoir of STEC currently known, and meat can become contaminated at different stages of the production chain. The aim of this work was to subtype and establish the epidemiological relatedness of non-O157 STEC strains isolated from ground beef and the environment in butcher shops before (evaluation stage, 2010–2011 period) and after (verification stage, 2013) implementing improvement actions. Sixty-eight non-O157 STEC strains were tested for *eae*, *saa*, *ehxA*, *iha*, *efa1*, *toxB*, *subAB*, *cdt-V*, *astA*, *aggR*, and *aaiC* genes, and stx_1 and stx_2 variants were determined. Pulsed-field gel electrophoresis (PFGE) was carried out with *Xba*I and *Xma*JI. From the 68 strains, 92.6%, 75.0%, 58.8%, 53.5%, 10.3%, 7.3%, and 4.4% were positive for *iha*, *ehxA*, *subAB*, *saa*, *cdt-V*, *astA*, and *eae*, respectively. All strains were *aggR/aaiC*-negative. PFGE showed that 19 strains grouped in 9 clusters and 41 showed unique *Xba*I patterns. During the evaluation stage (2010–2011), we identified clonal strains in different samples, circulating clones in different butcher shops, and more than one different strain in the same butcher shop. The bovine origin of meat and its manufacturing process could not ensure the total absence of all non-O157 STEC serotypes in this foodstuff. Most strains isolated during the evaluation (2010–2011) and verification (2013) stages did not exhibit a genotypic profile associated with human disease. It is necessary to conduct periodic reviews of the new epidemiological information and verify that the analyses of non-O157 STEC in food are appropriate to identify strains affecting the population.

Keywords: molecular subtyping, PFGE, STEC, butcher shops

Introduction

Shiga toxin–producing *Escherichia coli* (STEC) are important emerging foodborne human pathogens that cause mild to bloody diarrhea, hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome (HUS) and can lead to death (Karmali *et al.*, 2010; Tukur *et al.*, 2014). *E. coli* O157:H7 is the serotype most frequently associated with large outbreaks and sporadic cases of HC and HUS in many countries (Leotta *et al.*, 2008; Atkinson *et al.*, 2012; EFSA, 2013). However, other STEC serogroups recognized by the World Health Organization (WHO) for their pathogenic potential (WHO, 1997) could have the same virulence markers as O157.

Non-O157 STEC strains are responsible for over 60% of the STEC infections in the United States of America; O26, O45, O103, O111, O121, and O145 are the serogroups most frequently associated with severe disease worldwide (Atkinson *et al.*, 2012; EFSA, 2013; Gould *et al.*, 2013). In Argentina, non-O157 STEC strains are responsible for 25.1% of STEC infections, and O145, O121, O26, O174, O111, and O8 are the main serogroups isolated from ill patients (Rivas *et al.*, 2010).

STEC strains are characterized by the presence of *stx* genes that codify for Shiga toxin (Stx), the main STEC virulence factor (Calderwood and Mekalanos, 1987; Etcheverria and Padola, 2013; Kruger and Lucchesi, 2015). Intimin, an adhesin encoded by the *eae* gene in the locus of enterocyte effacement pathogenicity island of the chromosome, is involved in gut colonization. Although it is not clear which combination of markers defines a pathogenic STEC strain, stx_2/*eae* is associated with a higher risk of more serious illness (EFSA, 2013).

In addition to *stx* and *eae*, STEC strains could harbor a complex set of genetic determinants, including other toxin

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and adherence genes such as *saa* (STEC autoagglutinating adhesin), *lpf* (long polar fimbriae), *iha* (IrgA homolog adhesin), *efa* (enterohemorrhagic *E. coli* factor for adherence), *toxB* (plasmid virulence gene of *E. coli* O157), *cdt-V* (cytolethal distending toxin), *subAB* (subtilase cytotoxin), *astA* (enteroaggregative *E. coli* heat-stable toxin 1), and *ehxA* (enterohemolysin) (USDA, 2012; Etcheverria and Padola, 2013; Franz *et al.*, 2015).

Ruminants are the main animal reservoir of STEC currently known. Although contaminated foodstuffs derived from cattle such as ground beef are responsible for human illness (Hussein, 2007; Martin and Beutin, 2011; EFSA and ECDC, 2015), outbreaks attributed to leafy vegetables, dairy products, fruits, and other meats are more severe than those caused by beef, probably due to a change in strain virulence and host susceptibility by patient age and sex (Heiman *et al.*, 2015).

Pathogen bacteria can be transferred to beef carcasses during slaughtering, dressing, chilling, or cutting at slaughterhouses, and from the equipment to meat at retail stores (Perez-Rodriguez *et al.*, 2010; Vogeleer *et al.*, 2014). While meat mincing machines and handlers contaminated with STEC have been associated with meat cross-contamination in retail stores (Papadopoulou *et al.*, 2012), pathogen cross-contamination between food and the retail store environment has been described as a factor responsible for increased foodborne disease risk (Sirsat *et al.*, 2014). In the Autonomous City of Buenos Aires, Argentina, zero-tolerance criteria to STEC in meat products from supermarkets and fast food shops were applied between 2004 and 2008.

The aim of this work was to subtype and establish the epidemiological relatedness of non-O157 STEC strains isolated from ground beef and the environment in butcher shops before and after implementing improvement actions.

Materials and Methods

In October 2010, a pilot program called ''Healthy Butcher Shops'' was conducted in the city of Berisso, Buenos Aires, Argentina (Leotta *et al.*, 2016). A comprehensive risk assessment was performed in 172 raw ground beef provided by 10 slaughterhouses (A–J) and 672 environmental samples collected from 86 butcher shops (B1–B86) during the evaluation (2010–2011) and verification (2013) stages of the study.

During 2011, to implement improvement actions in butcher shops, we performed collective training meetings for butchers, customized trainings for handlers, and individual counseling at the stores, providing recommendations about facilities, good manufacturing practices, sanitation standard operating procedures, raw food handling, and meat preservation (Leotta *et al.*, 2016). Sixty-eight non-O157 STEC were isolated from ground beef and environmental samples (meat tables, knives, mincing machines, and manipulator hands; Leotta *et al.*, 2016). All strains were stored at −70°C in the strain collection of IGEVET (Instituto de Genética Veterinaria ''Ing. Fernando Noel Dulout,'' UNLP-CONICET LA PLATA) for further characterization.

Strains were grown in 4 mL brain heart infusion (BHI) broth (Biokar, Zac de Ther, France) at 37°C ± 1°C for 18–24 h. This culture was streaked onto BHI agar plates and incubated overnight at 37°C ± 1°C. A single colony from each strain was selected and grown overnight in 4 mL BHI broth at 37°C ± 1°C.

All strains were tested for the presence of the *eae*, *saa*, *ehxA*, *iha*, *efa1*, *toxB*, *subAB*, *cdt-V*, and *astA* genes (Galli *et al.*, 2010). The *aggR* and *aaiC* genes were detected by real-time PCR (ISS, 2013). The stx_1 variant was determined with primers stx1a-F1, stx1a-R1, stx1c-F1, stx1c-R1, stx1d-F1, and stx1d-R1 (Scheutz *et al.*, 2012). Genotyping of the stx_2 variant was performed with primers VT2-c, VT2-d, VT2v-1, and VT2v-2 by restriction fragment length polymorphism analysis of the DNA fragments obtained by PCR (Tyler *et al.*, 1991).

Molecular subtyping of non-O157 STEC strains was performed with pulsed-field gel electrophoresis (PFGE) using the 1 d (24–26 h) PulseNet standardized laboratory protocol (CDC, 2013). Restriction digestion of DNA in agarose plugs was carried out with *Xba*I and *Xma*JI (*Bln*I) as primary and secondary enzymes, respectively (Thermo Scientific). PFGE images of gels were obtained by MaestroGen slider® imager (Maestrogen, Inc., Nevada). TIFF image analysis was carried out with BioNumerics, version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) using the dice coefficient and the unweight pair group method with arithmetic mean (UPGMA) to generate dendrograms with 1.5% band matching tolerance. Two or more isolates were grouped in a cluster when they showed identical *Xba*I-PFGE pattern (100% similarity).

Results

Non-O157 STEC strains harbored different variants of the Shiga toxin gene, namely $stx_{2c(vh\text{-}b)}$ ($N=23$), stx_{2a} ($N=22$), stx_{1a}/stx_{2a} ($N=12$), $stx_{2a}/stx_{2c(vh\text{-}b)}$ ($N=4$), $stx_{2c(vh\text{-}a)}$ ($N=3$), stx_{1a} ($N=3$), and $stx_{1a}/stx_{2c(v\text{-}hb)}$ ($N=1$) (Table 1).

All strains were *eae*-negative (95.6%), except for three that were *eae*-positive (4.4%). The *ehxA* gene was carried by 51 isolates (75.0%). Forty-four *ehxA*-positive strains were *saa*-positive as well, whereas two *saa*-positive strains were *ehxA*-negative. The most prevalent putative adhesin was the one encoded by the *iha* gene, where 63 strains were positive (92.6%).

Gene sequences related to *subAB*, *cdt-V*, and *astA* toxins were present in 40 (58.8%), seven (10.3%), and five (7.3%) strains, respectively. One *subAB*-positive strain was also *eae*-positive and belonged to serotype O109:H25. The *astA*-positive strains belonged to serotypes O174:H21 ($N=3$) and O178:H19 ($N=1$), whereas one strain was nontypeable. None of the O174:H21 *astA*-positive strains could be subtyped by PFGE because of bacterial lysis. All strains were *aggR/aaiC*-negative. Only O26:H11 (*eae*-positive) harbored the *efa1* and *toxB* genes, while the rest of the strains were *efa1* and *toxB*-negative. The complete genotypic characterization is presented in Table 1.

Sixty-eight non-O157 STEC isolates were analyzed by *Xba*I-PFGE. Eight strains were excluded from the analysis because their DNA was degraded three times, even when thiourea was added to the running buffer (Table 1). The *Xba*I-PFGE UPGMA dendrogram is shown in Figure 1. Fifty *Xba*I-PFGE patterns were obtained, with a 54.8% similarity. Nineteen strains grouped in 9 clusters and 41 strains showed unique *Xba*I-PFGE patterns. Clusters I, II, III, IV, V, and VIII grouped strains from the 2010 to 2011 evaluation stage, whereas clusters VI, VII, and IX included strains of the 2013 verification stage.

Strain clusters were as follows: I, one strain from ground beef and one from meat mincing machine of B85; II, one

TABLE 1. SEROTYPE, *XBAI*-PFGE CLUSTER, GENOTYPES, SOURCE, AND STAGE OF 68 NON-O157 STEC STRAINS ISOLATED FROM BUTCHER SHOPS

Serotype (N)	*ID*	Xba*I-PFGE cluster*	*Genotype*	*B*	*Stage*	*Sample type*
O8:H19 (*N*=7)	1	II	*saa/ehxA/iha/stx*$_{2a}$	5	E	Manipulators hands
	2	III	*saa/ehxA/iha/stx*$_{1a}$/*stx*$_{2a}$	23	E	Meat table
	3			29	E	Knife
	4			57	E	Meat table
	5	-	*ehxA/iha/stx*$_{1a}$/*stx*$_{2a}$	20	E	Ground beef
	6	V	*ehxA/stx*$_{2a}$	20	E	Ground beef
	7			83		Ground beef
O21:H21 (*N*=1)	8	-	*saa/iha/stx*$_{1a}$	58	V	Mincing machine
O26:H11 (*N*=1)	9	-	*eae/efa/toxB/ehxA/iha/stx*$_{1a}$	87	V	Ground beef
O41:H14 (*N*=1)	10	-	*iha/stx*$_{2c(vh-b)}$	41	E	Ground beef
O44:HNT (*N*=1)	11	-	*subAB/stx*$_{2a}$	11	E	Meat table
O64:H20 (*N*=1)	12	-	*saa/ehxA/iha/stx*$_{2c(vh-b)}$	53	V	Ground beef
O79:H19 (*N*=2)	13	IV	*saa/ehxA/iha/subAB/stx*$_{2a}$/*stx*$_{2c(vh-b)}$	27	E	Meat table
	14		*saa/ehxA/iha/stx*$_{2c(vh-b)}$	63		Mincing machine
O91:H21 (*N*=1)	15	-	*saa/ehxA/iha/subAB/cdt-V/stx*$_{2a}$	70	V	Ground beef
O109:H25 (*N*=1)	16	-	*eae/ehxA/iha/subAB/stx*$_{2a}$	49	V	Mincing machine
O113:H21 (*N*=3)	17	I	*saa/ehxA/iha/subAB/cdt-V/stx*$_{1a}$/*stx*$_{2a}$	85	E	Ground beef
	18					Mincing machine
	19	-	*saa/ehxA/iha/subAB/cdt-V/stx*$_{2a}$	82	V	Ground beef
O116:H21 (*N*=2)	20	-	*saa/ehxA/iha/subAB/stx*$_{22c(vh-b)}$	33	V	Meat table
	21	-	*saa/ehxA/iha/subAB/cdt-V/stx*$_{2a}$	51	E	Knife
O116:H49 (*N*=1)	22	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	57	V	Manipulators hands
O130:H11 (*N*=1)	23	-	*iha/subAB/stx*$_{1a}$/*stx*$_{2a}$	24	V	Meat table
O130:H21 (*N*=1)	24	-	*saa/ehxA/iha/subAB/cdt-V/stx*$_{2a}$	62	E	Knife
O141:H49 (*N*=2)	25	IX	*saa/ehxA/iha/subAB/stx*$_{2c(vh-b)}$	49	V	Ground beef
	26	-	*saa/ehxA/iha/subAB/stx*$_{1a}$/*stx*$_{2c(vh-b)}$	63	V	Meat table
O163:HNM (*N*=1)	27	N/A	*saa/iha/subAB/stx*$_{2a}$	17	E	Ground beef
O171:H14 (*N*=2)	28	VII	*saa/ehxA/iha/subAB/stx*$_{2c(vh-b)}$	61	V	Meat table
	29		*saa/iha/subAB/stx*$_{2c(vh-b)}$			Mincing machine
O174:H21 (*N*=4)	30	-	*iha/stx*$_{2v-hb}$	46	E	Ground beef
	31	N/A	*iha/astA/stx*$_{2v-hb}$	40	E	Mincing machine
	32	N/A	*iha/astA/cdt-V/stx*$_{2v-hb}$	74	V	Manipulator hand
	33	N/A	*iha/astA/cdt-V/stx*$_{2v-hb}$	85	V	Knife
O174:H28 (*N*=6)	34	-	*saa/ehxA/iha/subAB/stx*$_{1a}$/*stx*$_{2a}$	47	V	Meat table
	35	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	10	E	Meat table
	36	-	*saa/ehxA/iha/subAB/stx*$_{2a}$/*stx*$_{2c(vh-b)}$	76	E	Meat table
	37	-	*saa/ehxA/iha/subAB/stx*$_{2a}$/*stx*$_{2c(vh-b)}$			Knife
	38	-	*saa/ehxA/iha/subAB/stx*$_{2a}$/*stx*$_{2c(vh-b)}$	44	V	Ground beef
	39	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	1	E	Mincing machine
O174:HNM (*N*=1)	40	N/A	*iha/stx*$_{2v-hb}$	73	E	Manipulator hand
O178:H19 (*N*=5)	41	II	*iha/stx*$_{2c(vh-a)}$	2	E	Mincing machine
	42	VI	*saa/ehxA/iha/subAB/stx*$_{1a}$/*stx*$_{2a}$	61	V	Meat table
	43					Knife
	44	-	*saa/ehxA/iha/subAB/stx*$_{1a}$/*stx*$_{2a}$	61	V	Ground beef
	45	-	*iha/stx*$_{2c(vh-a)}$	32	E	Mincing machine
ONT:H7 (*N*=6)	46	-	*iha/stx*$_{2c(vh-a)}$	77	V	Meat table
	47	-	*ehxA/stx*$_{2a}$	23	V	Meat table
	48	VIII	*iha/subAB/stx*$_{2c(vh-b)}$	81	E	Ground beef
	49		*iha/stx*$_{2c(vh-b)}$	82		Meat table
	50	-	*iha/stx*$_{2c(vh-b)}$	59	E	Mincing machine
	51	-	*iha/astA/stx*$_{2c(vh-b)}$	81	E	Meat table
ONT:H18 (*N*=1)	52	-	*saa/ehxA/stx*$_{1a}$/*stx*$_{2a}$	29	E	Mincing machine
ONT:H19 (*N*=7)	53	-	*saa/ehxA/iha/subAB/stx*$_{1a}$	47	V	Knife
	54	-	*saa/ehxA/iha/subAB/stx*$_{2c(vh-b)}$	81	V	Meat table
	55	-	*saa/ehxA/iha/subAB/stx*$_{2(vh-b)}$	57	V	Ground beef
	56	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	74	V	Mincing machine
	57	-	*saa/ehxA/ihastx*$_{1a}$/*stx*$_{2a}$	20	E	Ground beef
	58	N/A	*saa/ehxA/iha/subAB/stx*$_{2a}$	44	V	Meat table
	59	N/A	*saa/ehxA/iha/subAB/stx*$_{2a}$	44	V	Mincing machine

(*continued*)

Table 1. (Continued)

Serotype (N)	*ID*	Xba*I-PFGE cluster*	*Genotype*	*B*	*Stage*	*Sample type*
ONT:H21 ($N=2$)	60	-	*iha//stx*$_{2c(vh-b)}$	59	E	Knife
	61	N/A	*iha/stx*$_{2v-hb}$	37	V	Manipulator hands
ONT:H28 ($N=2$)	62	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	24	V	Knife
	63	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	36	V	Ground beef
ONT:H49 ($N=1$)	64	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	44	V	Meat table
O44:HNT ($N=1$)	65	IX	*saa/ehxA/iha/stx*$_{2c(vh-b)}$	49	V	Ground beef
ONT:HNM ($N=1$)	66	-	*saa/ehxA/iha/stx*$_{2c(vh-b)}$	75	V	Manipulators hands
ONT:HNT ($N=2$)	67	-	*saa/ehxA/iha/subAB/stx*$_{2a}$	79	E	Knife
	68	-	*saa/ehxA/iha/subAB/stx*$_{2c(vh-b)}$	84	V	Ground beef

-, strain that showed unique *Xba*I-PFGE pattern; B, Butcher shop; E, Evaluation stage (2010–2011); ID, strain identification number; *N*, number of strains isolated; N/A, not applicable (strain excluded from PFGE analysis due to DNA degradation); PFGE, Pulsed-field gel electrophoresis; S, Sampling stage; STEC, Shiga toxin–producing *Escherichia coli*; V, Verification stage (2013).

strain from manipulator hands of B5 and one from mincing machine of B2; III, one strain from meat table of B23, one from knife of B29, and one from meat table of B57 (the meat supplier of these three butcher shops was slaughterhouse A); IV, one strain from meat table of B27 and one from mincing machine of B63 (the meat supplier for both butcher shops was slaughterhouse A); V, one strain from ground beef of B20 and one from ground beef of B83; VI, one strain from meat table and one from knife of B61; VII, one strain from meat table and one from mincing machine of B61; VIII, one strain from ground beef of B81 and one from meat table of B82; and IX, two strains from ground beef of B49.

Strain ID44 isolated from ground beef of B61 (Fig. 1) showed one band of difference with strains of cluster VI isolated from meat table and knife of the same butcher shop. Due to the epidemiological relationship between these strains, they could be considered as a clone with a common origin (Tenover *et al.*, 1995). Strains ID 36 and ID 37 isolated from meat table and knife of B76, respectively, showed one band of difference between them (Fig. 1), and could be considered clones (Tenover *et al.*, 1995). Strains from cluster II and VII were distinguished by cutting with *Bln*1-PFGE.

Discussion

The principle of the bacterial subtyping approach is to compare the distribution of subtypes in potential sources (e.g., animals and food) with the subtype distribution in humans (EFSA, 2013). In this work, we investigated the prevalence of the new scheme of virulence genes proposed by the EFSA plus four putative adhesins and three toxin genes in 68 non-O157 STEC strains isolated from 86 Argentinian butcher shops in a previous study (Leotta *et al.*, 2016). The strains were also molecularly subtyped to compare the distribution of potential clones.

According to the EFSA (EFSA, 2013), there is not a unique combination of markers that define pathogenic STEC strains. However, *stx*$_2$/*eae* and *stx*$_2$/*aaiC*/*aggR* strains were associated with a higher potential risk of causing severe illness than other combinations of virulence genes (EFSA, 2013). In Argentina, 94.3% of STEC isolates from acute diarrhea, bloody diarrhea, or HUS cases harbored *stx* and *eae* genes (Rivas *et al.*, 2010).

In this study, all non-O157 STEC strains were *aaiC*/*aggR*-negative and 96.5% were *eae*-negative. Although most of the strains isolated corresponded to serotypes associated with illness in Argentina, they did not exhibit a genotypic profile associated with human disease, probably accounting for the absence of HUS cases in Berisso during the 2010–2013 study period (Galli *et al.*, 2016; Leotta *et al.*, 2016). Epidemiological surveillance should be intensified with the aim of identifying emerging strains with new pathogenic potential and determining whether they are associated with foodstuffs.

Among the *eae*-positive strains showing a genotypic profile associated with severe disease, we found one O26:H11 and one O109:H25 strain. O26:H11 (*stx*$_1$/*eae*) was the only strain showing a close association of the *efa* and *toxB* genes with intimin-positive strains. It also exhibited a highly potential risk of causing diarrhea or HUS/HC, as was described in previous studies (Cergole-Novella *et al.*, 2007). Strain O109:H25 isolated from mincing machines was *cdt-V* and *subAB*-positive.

Several authors proposed the possible role of the CDT-V and SubAB toxins in the infection pathogenesis caused by STEC (Bielaszewska *et al.*, 2004; Talbot *et al.*, 2005). Cergole-Novella *et al.* (2007) and Galli *et al.* (2010) observed these toxin genes in intimin-negative strains. However, in the present study, *subAB* was found in one O109:H25 *eae*-positive strain. Despite this serotype was previously associated with human and animal origin (Beutin *et al.*, 2004; Krause *et al.*, 2005), none of those strains carried the *subAB* gene. Consequently, the potential clinical relevance is also unknown.

Food equipment has been recognized as an important vehicle of contamination throughout the meat supply chain (Gounadaki *et al.*, 2008; Perez-Rodriguez *et al.*, 2010). During the evaluation stage (2010–2011), clonal non-O157 STEC strain in ground beef and mincing machine of B85 (cluster I) was detected by *Xba*I-PFGE. The poor sanitation of the mincing machine could have been the origin of ground beef contamination.

Biofilm presence and transference of pathogenic bacteria between food and utensils and equipment and handlers have been previously described in different stages of the meat production chain (Phang and Bruhn, 2011; Papadopoulou *et al.*, 2012; Vogeleer *et al.*, 2014). In addition, circulating clones were found in different butcher shops, such as those grouped in clusters III, IV, V, and VIII. Some of these clusters were composed of strains from butcher shops with the same meat supplier, and the source of contamination could have been at the slaughterhouse. Futures studies of meat suppliers could confirm whether they are a source of STEC contamination.

During the 2010–2011 evaluation stage, more than one different non-O157 STEC strain in B20, B29, and B81 was

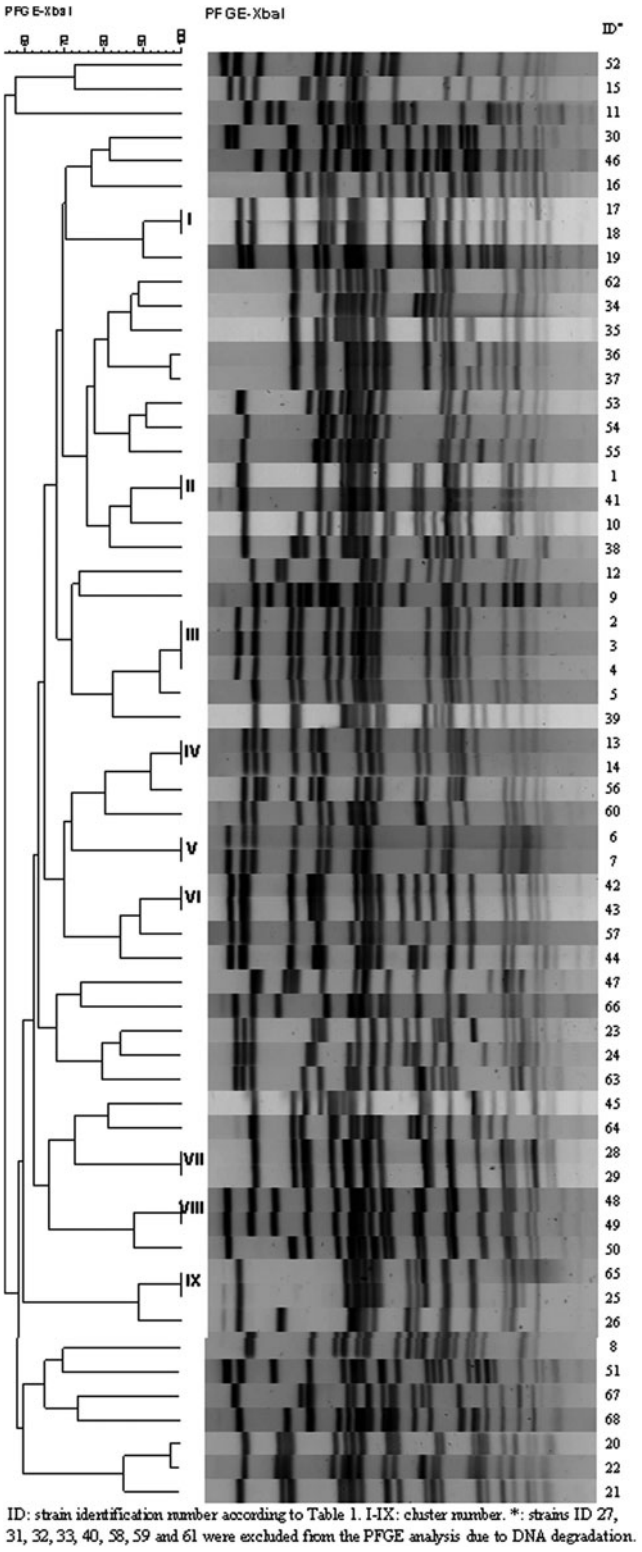

FIG. 1. *XBa*I-PFGE UPGMA dendrogram, sample type, sampling stage, serotype and genotype of 60 non-O157 STEC strains isolated from butcher shop. PFGE, pulsed-field gel electrophoresis; STEC, Shiga toxin–producing *Escherichia coli*; UPGMA, unweight pair group method with arithmetic mean.

identified. The presence of several nonclonal strains in the same butcher shop, even in the same sample, could be due to different sources of contamination such as slaughterhouse, transport, handlers, water, and vectors (Persad and LeJeune, 2014; Vogeleer *et al.*, 2014).

After the implementation of improvement actions, the percentage of STEC strains corresponded to the main non-O157 serotypes associated with illness cases in Argentina. Such percentage was reduced from 43.9% to 13.5% (Leotta *et al.*, 2016), and none of the clonal strains observed at this stage were the same as those isolated during the evaluation stage.

The lack of a defined combination of virulence factors required for STEC-associated clinical infection is not conclusive (EFSA, 2013). From the first published report of STEC serotypes in 1980, 1152 different serotypes have been described (Bettelheim and Goldwater, 2014). Most of them are *eae/aaiC/aggR*-negative and belong to serogroups other than O157, O26, O103, O145, O111, and O121. Thus, the prevailing uncertainty lies in their ability to cause disease or not (EFSA, 2013). We consider that due to the bovine origin of meat and its manufacturing process, it is impossible to assure the total absence of all non-O157 STEC serotypes in this foodstuff. In addition, there are no available methodologies for the isolation of all STEC serotypes in food (Brusa *et al.*, 2016).

Conclusion

Zero-tolerance criteria to non-O157 STEC in ground beef and environmental samples were applied in the isolation of all STEC strains from butcher shops of Berisso, Argentina. However, most strains isolated during the evaluation (2010–2011) and verification (2013) stages of the study did not exhibit a genotypic profile associated with human disease. These results indicate that the efforts to apply zero-tolerance criteria to non-O157 STEC in meat without a risk analysis that includes food and consumers would be excessive. Cross and multiple contamination in butcher shops as well as of circulating clones among butcheries in the city of Berisso were identified using PFGE. In this sense, slaughterhouses were recognized as a possible common source of STEC contamination in butcher shops.

Thus, studies of meat suppliers could be useful to know more about their importance as a source of contamination in the meat production chain. Strengthening epidemiological surveillance, based on molecular analysis, could give an insight into the clinical implications of virulence genes and allow the classification of STEC strains more efficiently according to risk. It would be necessary to conduct periodic reviews of the new epidemiological information and verify that the analyses of non-O157 STEC performed in foods are appropriate to identify strains affecting the population. The continuous work of all members of the food chain will allow a better approach to know the prevalence of non-O157 STEC strains in foodstuffs affecting both inhabitants and the epidemiological relationship between patients and meat.

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Disclosure Statement

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