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Subtyping of *Escherichia coli* O157:H7 Strains Isolated from Human Infections and Healthy Cattle in Argentina

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Abstract

Shiga toxin–producing *Escherichia coli* (STEC) cause nonbloody (NBD) and bloody diarrhea (BD), and hemolytic uremic syndrome (HUS). Cattle have been described as their main reservoir. STEC O157:H7 is recognized as the predominant serotype in clinical infections, but much less is known about the dominant subtypes in humans and animals or their genetic relatedness. The aims of this study were to compare the STEC O157 subtypes found in sporadic human infections with those in the bovine reservoir using *stx*-genotyping, phage typing, and *Xba*I–pulsed-field gel electrophoresis (PFGE), and correlate the subtypes with the severity of clinical manifestations. The 280 STEC O157:H7 strains collected included in this study were isolated from HUS ($n=122$), BD ($n=69$), and NBD ($n=30$) cases, and healthy carriers ($n=5$), and from bovines ($n=54$) in the abattoirs. The *stx*-genotyping showed that $stx_2/stx_{2c(vh\text{-}a)}$ was predominant in human (76.1%) and in bovine strains (55.5%), whereas the second more important genotype was stx_2 (20.8%) in human and $stx_{2c(vh\text{-}a)}$ (16.7%) in cattle strains. In human strains, PT4 (37.6%), PT49 (24.3%), and PT2 (18.6%) were the most frequent PTs (80.5%). In bovine isolates, PT2 (26%), PT39 (16.7%), and PT4 and PT49 (11.1% each) were predominant. By *Xba*I-PFGE, all 280 strains yielded 148 patterns with 75% similarity, and 169 strains were grouped in 37 clusters. Identical PT-PFGE-*stx* profile combinations were detected in strains of both origins: PT4-AREXH01.0011-$stx_2/stx_{2c(vh\text{-}a)}$ (12 humans and one bovine), PT4-AREXH01.0543-$stx_2/stx_{2c(vh\text{-}a)}$ (one human and four bovines), PT2-AREXH01.0076-$stx_2/stx_{2c(vh\text{-}a)}$ (one human and four bovines), PT49-AREXH01.0175-$stx_2/stx_{2c(vh\text{-}a)}$ (seven humans and one bovine), and PT49-AREXH01.0022-$stx_2/stx_{2c(vh\text{-}a)}$ (seven humans and one bovine). No correlation was found among the *stx*-genotypes, the phage type, and the clinical symptoms.

Introduction

Shiga toxin–producing *Escherichia coli* (STEC) are important foodborne pathogens that cause nonbloody (NBD) and bloody diarrhea (BD), and hemolytic uremic syndrome (HUS) in humans (Griffin and Tauxe, 1991). STEC O157:H7 is the dominant serotype associated with sporadic cases and outbreaks in different parts of the world (Rangel *et al.*, 2005; Pennington, 2010).

Cattle have been recognized as the main reservoir of *E. coli* O157 worldwide (Gyles, 2007), including Argentina (Masana *et al.*, 2010; Tanaro *et al.*, 2010). The main vehicles for acquiring the infection are the consumption of contaminated food, especially undercooked ground beef, fresh produce, water, and direct contact with infected persons or animals (Caprioli *et al.*, 2005).

Disease-associated human isolates of *E. coli* O157:H7 are characterized by the presence of specific sets of virulence genes, including those encoding Shiga toxins (stx_1, stx_2), intimin (*eae*), and hemolysin (*ehxA*) (Karmali, 2004).

In Argentina, post-enteric HUS is endemic, and approximately 400 new cases are reported annually, with an estimated rate of 12 cases per 100,000 children under 5 years old in 2009 (Rivas *et al.*, 2011), and STEC O157 is the serogroup most commonly identified (Rivas *et al.*, 2006).

There are a number of genotyping methods used for epidemiological studies of STEC O157, such as polymerase chain reaction (PCR) typing of several virulence factors, lineage-specific polimorphism assay using six markers (LSPA-6), restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE), and variable number tandem repeat analysis (MLVA) (Thomson-Carter, 2001; Yang *et al.*,

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2004; Hyytia-Trees *et al.*, 2006). Some studies have reported differences in the genotype and virulence markers of STEC O157 strains from human patients and cattle (Eklund *et al.*, 2002; Mora *et al.*, 2004; Roldgaard *et al.*, 2004; Barker *et al.*, 2007; Nakamura *et al.*, 2008; Aspán and Erikson, 2010; Lee *et al.*, 2011; Kawano *et al.*, 2012).

In this study, PFGE, phage typing, and *stx*-PCR-RFLP analysis of DNA fragments obtained by PCR were used to compare the STEC O157 subtypes found in sporadic human infections with those in the Argentine bovine reservoir, and to correlate the subtypes with the severity of clinical manifestations.

Methods

Bacterial strains

A total of 280 STEC O157 strains were included in this study. Between November 2006 and April 2008, 226 strains received at the National Reference Laboratory as part of the surveillance of HUS and diarrheal diseases were studied. The strains were isolated from HUS ($n=122$), BD ($n=69$), NBD ($n=30$) cases, and healthy carriers ($n=5$). For comparison purposes, 54 STEC O157 strains isolated from healthy beef cattle in a survey carried out in beef abattoirs during the same period (Masana *et al.*, 2010) were included as representative of the Argentine bovine reservoir.

Phenotypic and genotypic characterization of isolates

Confirmation of isolates as *E. coli* was performed through biochemical tests (Ewing, 1986) and serotyping (Ørskov and Ørskov, 1984) using the somatic and flagellar antisera provided by the Instituto Nacional de Producción de Biológicos–ANLIS "Dr. Carlos G. Malbrán."

Antimicrobial susceptibility to amikacin, ampicillin, ciprofloxacin, cloramphenicol, colistin, gentamicin, nalidixic acid, nitrofurantoin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole was established by standard methods (CLSI, 2011).

In all isolates, stx_1, stx_2, and rfb_{O157} genes were detected by a multiplex PCR as described by Leotta *et al.* (2005), whereas *eae*, *ehxA*, and $fliC_{H7}$ genes were detected by PCR as described by Karch *et al.* (1993), Schmidt *et al.* (1995), and Gannon *et al.* (1997), respectively.

To determine Stx production, cytotoxicity assays on Vero cells were performed (Karmali *et al.*, 1985). Enterohemolysis was determined on sheep blood agar plates (Beutin *et al.*, 1989).

Subtyping of isolates

The analysis of stx_1 variants was conducted according to Zhang *et al.* (2002). Genotyping of stx_2 variants was done by RFLP analysis of the B-subunit-encoding DNA fragments obtained by PCR (Tyler *et al.*, 1991).

Phage typing was performed with the method described by Ahmed *et al.* (1987) and extended by Khakhria *et al.* (1990), employing a set of 16 phages provided by the Canadian Centre for Human and Animal Health, Winnipeg, Manitoba, Canada, which allow the differentiation of 87 phage types (PTs).

PFGE protocols and data analysis of *E. coli* O157:H7 isolates were performed using the restriction endonucleases *Xba*I and *Bln*I (Promega, Madison, WI) following the standardized PulseNet methods from the Centers for Disease Control (Ribot *et al.*, 2006). The Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA) were used to generate dendrograms with 1.5% tolerance values. The strains were grouped in a cluster when they showed identical *Xba*I-PFGE pattern (100% similarity).

Statistical analysis

The statistical analysis of the frequency of *stx*-genotypes and PTs between human and cattle strains was performed by the two-tailed Fisher's exact test, using InStat version 3.05 (GraphPad Software, San Diego, CA). A p-value of <0.05 was considered statistically significant.

Results

Characterization of isolates

All 280 STEC O157:H7 strains were non-sorbitol-fermenting and β-D-glucuronidase negative. Among the strains of both origin, the biotype C (rhamnose+/dulcitol+) was predominant (93.6%), whereas 14 (5%) strains (12 humans and two bovines) belonged to biotype B (rhamnose−/dulcitol+), and four (1.4%) human strains belonged to biotype D (rhamnose+/dulcitol−).

All human and bovine strains harbored *eae*, *ehxA*, $rfbO_{157}$, and $fliC_{H7}$ genes. Most of the strains that carried the *ehxA* gene were hemolytic on sheep blood agar (95%); however, this activity was not detected in 14 strains of bovine origin. Expression of toxicity on Vero cells assays and flagella presence by slide agglutination was demonstrated in all isolates. Two hundred and nineteen (96.9%) of 226 human strains and 48 (89%) of 54 bovine strains were susceptible to all antibiotics assayed.

Subtyping of STEC O157 isolates

All 280 strains harbored the stx_2 gene, and 14 (5%) also carried the stx_1 gene. The *stx*-genotype analysis revealed that $stx_2/stx_{2c(vh\text{-}a)}$ was predominant (72.1%) in strains of both origin, with a higher frequency in human (76.1%) than in bovine (55.5%) strains ($p<0.05$). The stx_2 variant was detected in both human (20.8%) and bovine (9.2%) strains. In human isolates, the $stx_{2c(vh\text{-}a)}$ variant was detected in a low frequency (0.9%), whereas in bovine strains it was the second *stx*-genotype found (16.7%) ($p<0.0001$). The stx_1 gene in combination with other *stx* genes was more frequently detected in animal (16.6%) than in human (2.2%) strains (Table 1).

A total of 20 PTs were identified among the 280 strains. Two hundred and fourteen (94.7%) human strains were categorized into 15 different PTs, and 12 (5.3%) strains belonged to the react but do not conform (RDNC) category of the current phage typing scheme. PT4 (37.6%), PT49 (24.3%), and PT2 (18.6%) were the most frequent (80.5%) PTs found. Bovine isolates were grouped into 12 PTs, and two (3.7%) strains were RDNC. PT2 (26%), PT39 (16.7%), and PT4 and PT49 (11.1% each) were predominant, representing 64.9% of the total. PT4 was strongly associated to human strains and PT39 to bovine strains ($p<0.0001$) (Table 2). PT14, PT24, PT36, PT37, PT40, PT47, PT50, and PT54 were only detected in human strains, whereas PT21, PT31, PT33, PT43, and PT51 were only found in bovine strains.

Relationships between *stx*-genotype and PT in STEC O157:H7 strains of both origins are shown in Table 3. In

Table 1. Frequency of *stx*-Genotypes of *Escherichia coli* O157:H7 Strains Isolated from Humans and Healthy Bovines

stx-*genotype*	*No. of strains (%)*		
	Total (n = *280)*	*Human* (n = *226)*	*Bovine* (n = *54)*
$stx_2/stx_{2c(vha)}$	202 (72.1)	172 (76.1)	30 (55.5)[a]
stx_2	52 (18.6)	47 (20.8)	5 (9.2)
$stx_{2c(vha)}$	11 (3.9)	2 (0.9)	9 (16.7)[a]
$stx_1/stx_2/stx_{2c(vha)}$	9 (3.2)	5 (2.2)	4 (7.4)
stx_1/stx_2	4 (1.4)	0	4 (7.4)[a]
$stx_1/stx_{2c(vha)}$	1 (0.4)	0	1 (1.8)
stx_2UT	1 (0.4)	0	1 (1.8)

[a]Frequencies between human and bovine strains are statistically significant ($p < 0.05$).
UT, untypeable.

human strains, the $stx_2/stx_{2c(vh\text{-}a)}$ genotype was mainly associated with PT49 (52/172, 30.2%), followed by PT4 (50/172, 29.1%) and PT2 (39/172, 22.7%), whereas in bovine strains this *stx*-genotype was mainly related to PT2 (12/30, 40%). It is important to highlight that the stx_2 genotype was strongly associated with PT4 (32/47, 68.1%) in human strains, and $stx_{2c(vh\text{-}a)}$ to PT39 (6/9, 66.7%) in bovine strains. All four stx_1/stx_2 animal strains belonged to PT21.

By *Xba*I-PFGE, all 280 STEC O157 strains generated 148 different patterns with at least 75% similarity. One hundred and sixty-nine strains were grouped in 37 clusters, and 111 strains showed unique patterns. Twenty-four clusters grouped exclusively human strains, eight were specific for bovine strains, and five clusters (III, VI, XVIII, XXIII, XXIX) included both human (38) and bovine (12) strains (Table 4).

Using the three subtyping techniques, the following PT-*Xba*I-PFGE-*stx* profile combinations were detected in strains of both origins: PT4-AREXH01.0011-$stx_2/stx_{2c(vh\text{-}a)}$ (12 humans and one bovine), PT4-AREXH01.0543-$stx_2/stx_{2c(vh\text{-}a)}$ (one human and four bovines), PT2-AREXH01.0076-$stx_2/stx_{2c(vh\text{-}a)}$ (one human and four bovines), PT49-AREXH01.0175-$stx_2/stx_{2c(vh\text{-}a)}$ (seven humans and one bovine), and PT49-AREXH01.0022-$stx_2/stx_{2c(vh\text{-}a)}$ (seven humans and one bovine) (Fig. 1).

Using *Bln*I as second enzyme, all human and bovine strains of PT49-AREXH01.0175-$stx_2/stx_{2c(vh\text{-}a)}$ and PT49-AREXH01.0022-$stx_2/stx_{2c(vh\text{-}a)}$ profiles were not discriminated. The bovine strain belonging to the PT4-AREXH01.0011-$stx_2/stx_{2c(vh\text{-}a)}$ profile combination showed identical *Bln*I-PFGE pattern with five of 12 human strains. Human strains belonging to PT4-AREXH01.0543-$stx_2/stx_{2c(vh\text{-}a)}$ and PT2-AREXH01.0076-$stx_2/stx_{2c(vh\text{-}a)}$ profile combinations could be discriminated from the bovine strains, but showed patterns with 91.9% and 89.5% similarity, respectively (Fig. 1).

Table 2. Frequency of Predominant Phage Types of *Escherichia coli* O157:H7 Strains Isolated from Humans and Healthy Cattle

Phage type	*No. of strains (%)*			
	Total (n = *280)*	*Human* (n = *226)*	*Bovine* (n = *54)*	P
PT2	56 (20)	42 (18.6)	14 (26)	NS
PT4	91 (32.5)	85 (37.6)	6 (11.1)	< 0.0001
PT39	10 (3.6)	1 (0.4)	9 (16.7)	< 0.0001
PT49	61 (21.8)	55 (24.3)	6 (11.1)	< 0.05
PT54	10 (3.6)	10 (4.4)	0	NS

Table 3. Relationship Between *stx*-Genotypes and Predominant Phage Types in *Escherichia coli* O157:H7 Strains of Human and Bovine Origin

stx-*genotype*	*Origin*	*No.*	*Phage type (%)*
$stx_2/stx_{2c(vha)}$	Human	172	PT49 (30.2), PT4 (29.1), PT2 (22.7)
	Bovine	30	PT2 (40), PT4 and PT49 (20, each one), PT26 (6.7)
stx_2	Human	47	PT4 (68.1), PT2 (6.4), PT47, PT49, and PT54 (4.3, each one)
	Bovine	5	PT2, and PT33 (40, each one), PT8 (20)
$stx_{2c(vha)}$	Human	2	PT8
	Bovine	9	PT39 (66.7), PT43 (22.2), PT8 (11.1)
$stx_1/stx_2/stx_{2c(vha)}$	Human	5	PT4 (60), PT26, and PT49 (20, each one)
	Bovine	4	PT10, PT26, PT31, PT39
stx_1/stx_2	Human	0	
	Bovine	4	PT21
$stx_1/stx_{2c(vha)}$	Human	0	
	Bovine	1	PT39
stx_2UT	Human	0	
	Bovine	1	PT33

UT, untypeable.

The analysis of the most frequent PTs and *stx*-genotypes revealed an equal distribution among strains associated with different clinical symptoms (Table 5). STEC O157 strains harboring $stx_2/stx_{2c(vh\text{-}a)}$ genes belonging to PT4, PT49, and PT2 were predominant.

Discussion

Previous studies indicate that the clinical outcome of STEC infection depends on the *stx* genotype of the infecting strain (Jelacic *et al.*, 2003; Persson *et al.*, 2007), and that there exists an increased risk for developing HUS when both stx_2 and *eae* genes are present (Böerlin *et al.*, 1999). By *stx*-genotyping, we found that the $stx_2/stx_{2c(vh\text{-}a)}$ genotype was predominant in strains isolated from both human (76.1%) and bovine (55.5%). Moreover, stx_2 was detected in human and bovine strains (20.8% vs. 9.2%), whereas the $stx_{2c(vh\text{-}a)}$ gene was predominant in the animal reservoir (16.7%) but not in humans (0.9%). The stx_1 gene was always detected together with other genes. The $stx_1/stx_2/stx_{2c(vh\text{-}a)}$ genotype was harbored by nine (3.2%) strains: five of them isolated from HUS ($n = 2$), BD ($n = 2$), and NBD ($n = 1$) cases.

A number of studies have documented that $stx_2/stx_{2c(vh\text{-}a)}$ genotype is more often associated with HUS than other stx_2 genotypes (Friedrich *et al.*, 2002; Eklund *et al.*, 2002). Our findings that $stx_2/stx_{2c(vh\text{-}a)}$ is prevalent in strains of both origins is relevant because this *stx*-genotype was also

Table 4. Distribution of *Escherichia coli* O157:H7 Strains from Human and Bovine Origin in XbaI-PFGE Clusters

Cluster	XbaI-*PFGE patterns*	stx-*genotypes*	*Phage type*	*Origin* (n)
I	AREXHX01.0390	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (5)
II	AREXHX01.0267	$stx_2/stx_{2c(vh\text{-}a)}$	49	Bovine (3)
III	**AREXHX01.0022**	$\boldsymbol{stx_2/stx_{2c(vh\text{-}a)}}$	**49**	**Human (7), bovine (1)**[a]
		$stx_{2c(vh\text{-}a)}$	4	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	14	Human (3)
IV	AREXHX01.0093	$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (2)
V	AREXHX01.0189	$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (2)
VI	**AREXHX01.0175**	$\boldsymbol{stx_2/stx_{2c(vh\text{-}a)}}$	**49**	**Human (7), bovine (1)**
VII	AREXHX01.0153	$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (10)
		stx_2	4	Human (2)
		$stx_2/stx_{2c(vh\text{-}a)}$	RDNC	Human (1)
VIII	AREXHX01.0049	stx_2	2	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	RDNC	Human (5)
IX	AREXHX01.0507	$stx_{2c(vh\text{-}a)}$	39	Bovine (6)
X	AREXHX01.0514	$stx_{2c(vh\text{-}a)}$	43	Bovine (2)
XI	AREXHX01.0200	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (2)
		stx_2	4	Human (5)
XII	AREXHX01.0193	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (2)
XIII	AREXHX01.0341	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (3)
XIV	AREXHX01.0038	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (2)
XV	AREXHX01.0469	stx_2	54	Human (1)
		stx_2	8	Human (1)
XVI	AREXHX01.0057	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (3)
		stx_2	4	Human (2)
		$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (2)
XVII	AREXHX01.0007	$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (2)
XVIII	**AREXHX01.0011**	$\boldsymbol{stx_2/stx_{2c(vh\text{-}a)}}$	**4**	**Human (12), bovine (1)**
		$stx_2/stx_{2c(vh\text{-}a)}$	RDNC	Bovine (1)
		stx_2	4	Human (5)
		$stx_2/stx_{2c(vh\text{-}a)}$	14	Human (1)
XIX	AREXHX01.0064	stx_2	4	Human (15)
		$stx_1/stx_2/stx_{2c(vh\text{-}a)}$	4	Human (2)
XX	AREXHX01.0012	stx_2	4	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (1)
XXI	AREXHX01.0086	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (1)
XXII	AREXHX01.0458	$stx_1/stx_2/stx_{2c(vh\text{-}a)}$	26	Bovine (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	26	Bovine (2)
XXIII	**AREXHX01.0543**	$\boldsymbol{stx_2/stx_{2c(vh\text{-}a)}}$	**4**	**Human (1), bovine (4)**
XXIV	AREXHX01.0058	$stx_2/stx_{2c(vh\text{-}a)}$	26	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	4	Human (1)
XXV	AREXHX01.0517	stx_2	33	Bovine (2)
		stx_2 UT	33	Bovine (1)
XXVI	AREXHX01.0454	$stx_2/stx_{2c(vh\text{-}a)}$	37	Human (1)
		$stx_{2c(vh\text{-}a)}$	8	Human (1)
XXVII	AREXHX01.0143	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (2)
XXVIII	AREXHX01.0095	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (4)
XXIX	**AREXHX01.0076**	$\boldsymbol{stx_2/stx_{2c(vh\text{-}a)}}$	**2**	**Human (1), bovine (4)**
XXX	AREXHX01.0001	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	24	Human (1)
		$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (1)
XXXI	AREXHX01.0427	stx_2	4	Human (1)
		$stx_1/stx_2/stx_{2c(vh\text{-}a)}$	4	Human (1)
XXXII	AREXHX01.0460	$stx_2/stx_{2c(vh\text{-}a)}$	2	Bovine (3)
XXXIII	AREXHX01.0013	$stx_2/stx_{2c(vh\text{-}a)}$	49	Human (1)
		stx_2	49	Human (1)
XXXIV	AREXHX01.0449	$stx_2/stx_{2c(vh\text{-}a)}$	2	Human (2)
XXXV	AREXHX01.0145	$stx_2/stx_{2c(vh\text{-}a)}$	54	Human (2)
XXXVI	AREXHX01.0545	$stx_2/stx_{2c(vh\text{-}a)}$	2	Bovine (2)
XXXVII	AREXHX01.0544	stx_1/stx_2	21	Bovine (4)

[a]Identical *Xba*I-PFGE-*stx*-PT profile combinations detected in strains of both origins are shown in boldface.
RDNC, react but does not conform to the current phage typing scheme; UT, untypeable.

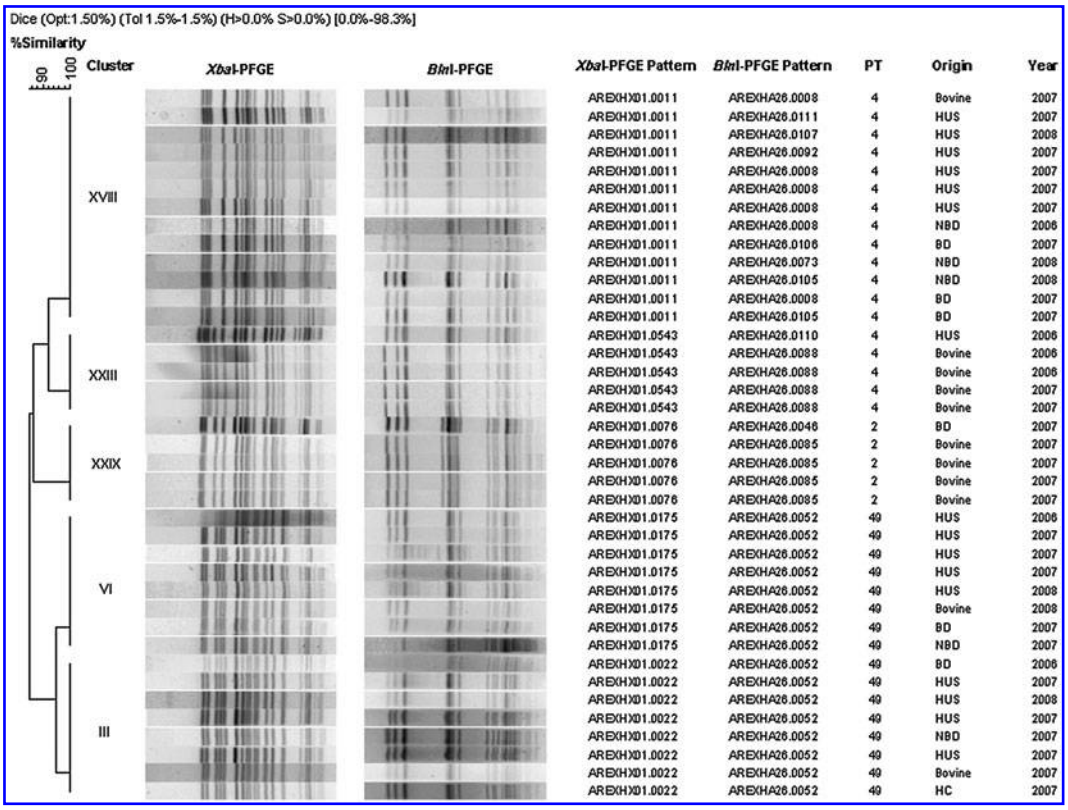

FIG. 1. Clonal relatedness by *Xba*I- and *Bln*I-PFGE, and phage types in human and bovine *Escherichia coli* O157:H7 *stx*2/*stx*2c (vh-a) strains with identical profile combinations. HUS, hemolytic uremic syndrome; BD, bloody diarrhea; NBD, nonbloody diarrhea; HC, healthy carriers.

predominant in other countries. In Sweden, Aspán and Erikson (2010) reported that STEC O157:H7 strains characterized as being PT4:$stx_2/stx_{2c(vh\text{-}a)}$ were the cause of most cattle-to-human transmitted STEC in the 1996–2002 period. In Finland, Eklund *et al.* (2002) found that 64% of human STEC O157 isolates carried stx_2/stx_{2c} genes, and the virulence profile PT2:$stx_2/stx_{2c}/eae/ehxA$ was significantly more frequently associated with HUS and BD than other profiles. Moreover, Manning *et al.* (2008) found that this *stx*-genotype was predominant among strains of the clade 8 lineage associated with two unusually severe outbreaks linked to fresh produce. Recent studies performed in Argentina using LSPA-6 to identify lineages (Yang *et al.*, 2004) and clade typing (Manning *et al.*, 2008) show that lineage I/II-clade 8 strains predominate in human isolates (>80%) and may explain the high incidence of HUS in our country (unpublished data).

Table 5. Relationship Between Predominant Phage Types, *stx*-Genotypes, and Clinical Symptoms

Phage type	stx-*genotype*	*Percentage by clinical symptom* HUS (n=122)	BD (n=69)	NBD (n=30)
PT4	$stx_2/stx_{2c(vha)}$	23.8	21.7	20.0
	stx_2	18.0	14.5	0
	$stx_1/stx_2/stx_{2c(vha)}$	0.8	1.4	3.3
PT49	$stx_2/stx_{2c(vha)}$	23.0	20.3	23.3
	stx_2	0	1.4	3.3
	$stx_1/stx_2/stx_{2c(vha)}$	0	1.4	0
PT2	$stx_2/stx_{2c(vha)}$	16.4	15.9	20.0
	stx_2	1.6	0	3.3
PT54	$stx_2/stx_{2c(vha)}$	4.1	4.3	0
	stx_2	0.8	0	3.3
PT14	$stx_2/stx_{2c(vha)}$	4.1	3.1	3.3

HUS, hemolytic uremic syndrome; BD, bloody diarrhea; NBD, nonbloody diarrhea.

In contrast, Nakamura *et al.* (2008) in Japan found that the predominant *stx*-genotype in human patients was stx_2 (68.7%), whereas the strains from asymptomatic carriers possessed only $stx_{2c(vh\text{-}a)}$ (47.9%), and the stx_1 gene was found in a few isolates (1.5%). Meanwhile, cattle harbored mainly $stx_{2c(vh\text{-}a)}$ (58.4%), followed by stx_2 (26.0%) and $stx_2/stx_{2c(vh\text{-}a)}$ (10.4%).

We found 20 different known PTs among the 280 isolates of STEC O157 studied, but only three types were predominant in human strains, PT4 (37.6%), PT49 (24.3%), and PT2 (18.6%), whereas four were predominant in bovine strains, PT2 (26%), PT39 (16.7%), and PT4 and PT49 (11.1% each). In Denmark, Roldgaard *et al.* (2004) found that PT2 (19%) and PT4 (28%) were also predominant in human isolates, but these PTs were only identified in 2% and 8%, respectively, of the bovines isolates. In the same study, PT8 and PT14 constituted approximately 35–40% of isolates from both origins. PT8 was responsible for 27% of human cases, and PT14 was the most predominant PT in cattle (22%). In the present study, PT8 was found in a very low frequency in human (1.3%) and bovine

(3.7%) isolates, and PT14 was only found in clinical isolates (3.5%). Rivas *et al.* (2006) found one PT8:$stx_2/stx_{2c(vh\text{-}a)}$ strain isolated from a BD case who developed HUS, and three PT14:$stx_2/stx_{2c(vh\text{-}a)}$ strains isolated from BD cases, during a prospective case-control study carried out in 2001–2002. Chinen *et al.* (2001) found 2/11 (18.2%) strains of PT14 harboring the $stx_2/stx_{2c(vh\text{-}a)}$ and $stx_{2c(vh\text{-}a)}$ genotypes, which were isolated from ground beef and fresh sausage, respectively, during a survey performed in 136 retail meats. Moreover, 2/20 (10%) *E. coli* O157:H7 strains characterized as PT8:$stx_2/stx_{2c(vh\text{-}a)}$ and PT14:$stx_1/stx_{2c(vh\text{-}a)}$ were isolated from undercooked beef burger during sampling procedures in fast food restaurants (Chinen *et al.*, 2009). In Spain (Mora *et al.*, 2004), Germany (Beutin *et al.* 2002), and Denmark (Roldgaard *et al.*, 2004), PT8 strains showed a high association with stx_1 and were mainly non-motile. In Argentina, all 280 strains were motile, and this could be another important virulence factor.

Between 1998 and 2004, PT21/28 was described as the most frequent PT in the United Kingdom, comprising over 50% of the positive cattle isolates and reported human cases, suggesting a link between bovine shedding and human infection (Pearce *et al.*, 2009). In Argentina, PT21/28 has never been found.

We found a marked overlap between the PT-*stx* profiles in strains isolated from cattle and those recovered from human diseases. The profiles PT49:$stx_2/stx_{2c(vh\text{-}a)}$ (30.2% vs. 20%), PT4:$stx_2/stx_{2c(vh\text{-}a)}$ (29.1% vs. 20%), and PT2:$stx_2/stx_{2c(vh\text{-}a)}$ (22.7% vs. 40%) were predominant in strains of both origins (Table 3). However, some PT-*stx*-genotype profiles were exclusively found in the bovine reservoir. These findings are consistent with the notion that only a subset of bovine O157:H7 is implicated in human disease.

In this study, it was not possible to establish a relationship between the *stx*-genotypes found in human strains and the clinical symptoms, probably due to the high rate of $stx_2/stx_{2c(vh\text{-}a)}$ genotype (>60%) observed in Argentina throughout the years (Rivas *et al.*, 2011). Other methods, such as *stx* expression and the amount of toxin produced, could be used to further relate the severity of the infections to particular subtypes.

The molecular surveillance of STEC has been implemented through PulseNet Latin America and The Caribbean. From 1988 to 2010, a total of 780 *Xba*I-PFGE patterns corresponding to 1,954 STEC O157 strains isolated from human infections ($n=1{,}693$), food ($n=185$), animals ($n=191$), and environmental samples ($n=35$) were included in the Argentine Database for O157.

In the present work, *E. coli* O157 strains showed a high degree of diversity. A total of 148 different *Xba*I-PFGE patterns were established among the 280 strains studied, with the strains that yielded identical patterns grouped in clusters. The five common *Xba*I-PFGE patterns identified in both human and bovine strains in the present study had been previously included in the National Database. These patterns corresponded to strains isolated from HUS ($n=120$), BD ($n=94$), NBD ($n=52$), cooked and uncooked ground meat ($n=18$), sausages ($n=2$), and water streams ($n=1$), indicating that they are widespread in human population, and are also detected in food and in the environment. Two of these common patterns, AREXH01.0011 and AREXH01.0022, are predominant in the Argentine Database of *E. coli* O157, representing 23.1% and 13.7%, respectively.

The AREXH01.0011 pattern is identical to SMI-H and EXH01.0047 patterns, described as predominant in Sweden and the United States, respectively. The Swedish type has also the other characteristics of the Argentine type, i.e., PT4 and $stx_2/stx_{2c(vh\text{-}a)}$ genes (Löfdahl, 2008). It would be of great value to perform the surveillance of this type more carefully in the rest of the world.

A total of 28 of 226 (12.4%) human strains showed identical PT-*Xba*I-PFGE-*stx* profile combinations as those found in 11 of 54 (20.4%) cattle strains. Some STEC strains of both origins could be discriminated using *Bln*I as second enzyme. Although they must be considered as different, they presented a certain degree of similarity that should be evaluated in the epidemiologic context. This overlap of human and bovine strains indicates that cattle are important reservoir for humans. It was possible to link 12% of reported human infections to the bovine reservoir by analyzing a very small proportion from the total cattle at slaughter in Argentina, estimated as approximately 21 million heads in the period under study (IPCVA, 2011).

The STEC prevalence in cattle and the high consumption of beef in Argentina are a risk for human population. Recently, a more extensive investigation on STEC O157 in nine selected beef exporting abattoirs of Argentina showed a prevalence of 4.1% (CI 95%, 5.6–2.9%) in the fecal content and 2.6% (CI 95%, 3.9–1.6%) in the carcasses of 811 bovines at slaughter (Masana *et al.*, 2010). From these data, and slaughter statistics, it could be roughly estimated that a proportion of around 38,000 bovines were contaminated with STEC O157 in their feces at slaughter per each reported human case. This should be taken into account to determine control and prevention guidelines aimed at reducing the burden of these diseases in our country.

Conclusion

This study showed that the use of phage typing, PFGE, and *stx*-genotyping techniques as epidemiological tools that may help to detect reservoirs, and establish temporal and geographical variations of newly emerging clones. However, other typing methods must be employed to relate the subtypes to the severity of the associated-illnesses.

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