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Original Articles

Non-O157:H7 Shiga Toxin–Producing *Escherichia coli* in Bovine Rectums and Surface Water Streams on a Beef Cattle Farm in Argentina

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Abstract

The purposes of this study were to detect non-O157 Shiga toxin–producing *Escherichia coli* (STEC) in bovine rectums and water in a beef cattle farm in Argentina, and to determine the pathogenic potential of the circulating strains. During the study, 292 rectal swabs from healthy animals and 79 environmental water samples were collected. The rectal swabs and one loop of the Moore swabs, enriched in *Escherichia coli* broth for 24 h at 37°C, were streaked on MacConkey agar plates and incubated overnight at 37°C. The isolates were characterized by biochemical tests and serotyped. Nonmotile STEC strains were typed for their H-specific (*fli*C) antigens by polymerase chain reaction (PCR). Isolates were characterized by detection of stx_1, stx_2, and their variants, *eae*, *ehx*A, and *saa* genes. Macrorestriction fragment analysis by pulsed-field gel electrophoresis (PFGE) was performed using the PulseNet standardized protocol. From 371 samples analyzed, 36.6% of rectal swabs and 34.2% of water samples were non-O157 STEC-positive by PCR, and 110 strains from rectal swabs, but only three from water, were isolated. The strains were grouped into 24 different serotypes, from which, O103:[H2] ($n=12$), O136:H12 ($n=8$), O178:H19 ($n=8$), and O103:NM ($n=5$) were most prevalent, representing 29.2% of the isolates. Predominant genotypes were $stx_1/eae/ehxA$ (16.8%) and $stx_2/saa/ehx$A (15.9%). PFGE analysis revealed 56 different patterns, with 65 strains grouped in 19 clusters of 100% similarity. Two STEC O124:H19 strains isolated from rectal swabs and water with a 5-month interval harbored the $stx_1/stx_2/saa/ehxA$ genotype, and showed an indistinguishable PFGE profile. By comparison, some *Xba*I-PFGE patterns identified in the present study were identical to the profiles of strains isolated from human, food, and animal sources included in the Argentine PulseNet database. By PCR, similar non-O157 detection rates were found in rectal swabs and water. However, the methodology for water samples needs to be improved, since only three strains from the total number of positive samples were recovered.

Introduction

Shiga toxin–producing *Escherichia coli* (STEC) have been recognized as human pathogens since Karmali *et al.* (1983) established their association with clinical cases of hemolytic uremic syndrome (HUS), a life-threatening complication characterized by hemolytic anemia, thrombocytopenia, and renal failure. STEC O157:H7/NM (STEC O157) is considered the prototype strain capable of producing severe foodborne illness outbreaks. However, the rising numbers of non-O157 STEC–associated illnesses reported by health services worldwide has driven more attention to the non-O157 STEC serotypes as a group of emerging pathogens of concern (Mathusa *et al.*, 2010). Argentina has the highest HUS rate globally: 13.9/100,000 children under age 5. Post-enteric HUS is considered the second cause of chronic kidney failure among children (Spizzirri *et al.*, 1997). According to the Argentine Database of the National Reference Laboratory (NRL) for HUS surveillance, non-O157 STEC strains accounted for approximately 30% of the STEC infections in the 2004–2009 period, with O-groups O145, O121, O26, O174, O111, and O103 being the most commonly associated with

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disease in order of frequency (Rivas *et al.,* 2011). Different epidemiologic investigations have associated non-O157 outbreaks to specific exposures to food and environmental water (Brooks *et al.,* 2005). In January 2011, the Centers for Disease Control and Prevention (CDC) reported the importance of the so-called rare non-O157 STEC that caused more than 113,000 cases of foodborne illness, whereas the annual incidence for *E. coli* O157:H7 was around 63,000 (Scallan *et al*., 2011). In Argentina, out of approximately 400 new cases of HUS cases reported annually, 60% are caused by *E. coli* O157:H7, while 40% are caused by non-O157. The detection of non-O157 STEC infections is limited to a few laboratories because no specific isolation media is available for this bacterial group. Thus, their true prevalence for human health is largely unknown (Smith *et al.,* 2001). The purposes of this study were to detect non-O157 STEC in bovine rectums and water in a beef cattle farm in Gualeguaychú, Argentina, and to determine the pathogenic potential of the circulating strains. The characterization of non-O157 STEC presented in this paper completes the previous survey report on STEC O157 (Tanaro *et al.,* 2010).

Materials and Methods

Between September 2005 and November 2006, 292 rectal swabs from healthy cattle and 79 water samples from two streams were collected. The sample collection used was the same as that described in a previous study (Tanaro *et al.,* 2010). Sample size was calculated using the Epi Info software (version 6.0), taking into account an estimated frequency of 10% and a precision of 1.5% at the 95% confidence level. Rectal swabs were plated directly *in situ* on MacConkey agar (Biokar Diagnostics, Beauvois, France) and incubated overnight. After incubation, the confluent growth zone and individual colonies were screened for stx_1, stx_2, and rfb_{O157} genes by a multiplex polymerase chain reaction (PCR) (Leotta *et al*., 2005). At least 30 presumptive *E. coli* colonies were selected from each MacConkey plate for PCR confirmation.

Moore swabs were placed in flasks with 100 mL of *Escherichia coli* broth (Biokar Diagnostics, Beauvois, France) and incubated overnight at 37°C. After incubation, a loop from each enrichment broth was streaked on MacConkey agar and processed as was described for rectal swabs.

Presumptive non-O157 STEC colonies from each PCR-positive fecal and water sample were isolated on Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Franklin Lakes, NJ), confirmed by multiplex PCR, and kept in Trypticase Soy Broth (TSB; Difco, Becton Dickinson) with 40% glycerol at −70°C for further phenotypic and genotypic characterization.

Phenotypic and genotypic characterization of the isolates

Confirmation of isolates as *E. coli* was performed through biochemical tests according to Ewing (1986). Serotyping was conducted by standard procedure of Ewing (1986), with somatic (O1-O181) and flagellar (H1-H56) antisera prepared at the Adolfo Lutz Institute (São Paulo, Brazil), with reference strains obtained from the *E. coli* and *Klebsiella* International Reference Centre (Copenhagen, Denmark). The H types of some nonmotile STEC strains were investigated for their H-type-specific (*fliC*) genes by restriction fragment length polymorphism analysis (RFLP) of the DNA fragments obtained by PCR as described by Machado *et al*. (2000). Enterohemolysis was determined on sheep blood agar plates according to Beutin *et al.* (1989). Isolates were characterized by detection of stx_1 and stx_2 genes by the multiplex PCR as mentioned above, while *eae, saa,* and *ehxA* genes were investigated as described by Karch *et al.* (1993), Paton and Paton (2002), and Schmidt *et al.* (1995), respectively.

Subtyping of the 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 (Pièrard *et al*., 1998; Tyler *et al*., 1991). Macrorestriction fragment analysis by pulsed-field gel electrophoresis (PFGE) was performed using the 24-h PulseNet standardized PFGE protocol for *E. coli* O157:H7 (Ribot *et al*., 2006). Restriction digestion of DNA was carried out with *Xba*I enzyme (Promega, Madison, WI). PFGE images of gels were obtained by Gel Doc 2000 (Bio-Rad, Hercules, CA). Analysis of TIFF images was carried out through the BioNumerics version 4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and the Unweighted Pair Group with Arithmetic Mean to generate dendrograms with 1.5% tolerance values.

Statistical analysis

The statistical analysis to find differences between the numbers of isolated strains in different seasons was made by differences between proportions, using the StatPlus 2009-5.7.8 program.

Results

Prevalence of non-O157 STEC strains

From the 371 samples analyzed, 107/292 (36.6%) rectal swabs (one per cow) and 27/79 (35.4%) water samples were PCR-positive for the presence of non-O157 STEC. A total of 113 strains were isolated, 110 from rectal swabs, and three from water samples. More than one serotype was detected in 3/292 (1%) animal samples (Table 1).

Figure 1 shows the number of samples analyzed and the PCR-positive rectal swabs and environmental water samples detected. The prevalence of non-O157 STEC in feces fluctuated with statistical differences ($p < 0.05$) from 52.7% (39/74) in winter to 37.5% (27/72) in autumn ($p = 0.03$), 36.1% (26/72) in summer ($p = 0.02$), and 20.3% (15/74) in spring seasons ($p = 0.0001$). There were also statistical differences between spring and autumn ($p = 0.01$), and spring and summer ($p = 0.02$), but there were no statistical differences between autumn and summer ($p > 0.05$). In water samples, the frequency was 42.9% (12/28) in spring, 37.5% (6/16) in autumn, 26.7% (4/15) in summer, and 25.0% (5/20) in winter seasons with non-statistical differences ($p > 0.05$).

Characterization of non-O157 STEC isolates

One hundred thirteen non-O157 STEC isolates were characterized by phenotypic and genotypic techniques, and serotyped, and 102 were analyzed by *Xba*I-PFGE. Most of the non-O157 strains were sorbitol fermenting (93.6%), and β-glucuronidase (86.7%) and enterohemolysin (56.6%) positive, and motile (82.3%).

Table 1. Prevalence of Non-O157 Shiga Toxin–Producing *Escherichia coli* in Cattle Rectums and Environmental Water

Sample category	*No. of samples analyzed*	*No. (%) of positive samples*	*95% CI*	*No. of strains isolated*	*No. (%) of samples with more than one serotype*
Rectal swabs	292	107 (36.6)	31.3–42.3	110	3 (1.0)
Water	79	27 (34.2)	24.4–45.1	3	—

95% CI, 95% confidence interval.

Among the 113 non-O157 STEC isolates, 71 belonged to 20 O-groups (O2, O7, O8, O15, O22, O79, O84, O91, O103, O107, O113, O116, O124, O130, O136, O141, O163, O174, O178, and O179), while 35 isolates were nontypeable (NT) and seven were rough (R). Twelve H antigens (H2, H7, H8, H11, H12, H16, H19, H21, H25, H27, H28, and H49) were determined in 90 strains, while 20 strains were nonmotile (NM) and three nontypeable (NT). Non-O157 STEC strains were grouped into 24 different serotypes, being O103:[H2] ($n=12$), O136:H12 ($n=8$), O178:H19 ($n=8$), and O103:NM ($n=5$), the most prevalent serotypes, representing 29.2% (33/113) of the total. Among nontypable somatic strains, ONT:H2 ($n=17$) was the most prevalent one (Table 2). It is interesting to notice that in three cattle the following STEC co-infections were detected: ONT:H2/O174:H28; ONT:H19/O130:H11; and ONT:H19/O103:[H2].

Eighty-four (74.3%) strains harbored the stx_2 gene and 47 (41.6%) the stx_1 gene. In the strains isolated from cattle, different variants were detected: stx_1 (26.4%), stx_2 (17.3%), $stx_{2c(vh\text{-}a)}$ (14.5%), stx_1/stx_2 (13.6%), $stx_{2c(vh\text{-}b)}$ (11.8%), $stx_{2c(vh\text{-}a)}/stx_{2c(vh\text{-}b)}$ (5.4%), $stx_2/stx_{2c(vh\text{-}b)}$ (4.5%), $stx_2/stx_{2c(vh\text{-}a)}$ (1.8%), $stx_1/stx_{2c(vh\text{-}b)}$ (1.8%), stx_{2NT} (1.8%), and $stx_1/stx_2/stx_{2c(vh\text{-}b)}$ (0.9%). In the three water strains, the detected *stx*-genotypes were stx_2, $stx_{2c(vh\text{-}a)}$, and stx_1/stx_2. Other virulence genes such as *eae* (18.6%), *ehx*A (56.6%), and *saa* (38%) were also determined. Seventeen different virulence genotypes were established, with $stx_1/eae/ehxA$ (19 strains), $stx_2/saa/ehxA$ (18 strains), $stx_1/stx_2/saa/ehxA$ (16 strains), $stx_{2c(vh\text{-}a)}$ (15 strains), $stx_{2c(vh\text{-}b)}$ (11 strains), and stx_1 (10 strains) being the most frequent (Table 3).

Twenty-one (18.6%) strains, of O103:[H2] ($n=12$), O103:NM ($n=5$), ONT:H21 ($n=2$), and ONT:HNT ($n=2$) serotypes, carried the *eae* and *ehx*A genes. Forty-three (38%) strains, belonging to ONT:H19 ($n=7$), O116:H21 ($n=4$), O178:H19 ($n=4$), O141:H49 ($n=3$), O179:H8 ($n=3$), O113:H21 ($n=2$), O113:HNM ($n=2$), O8:H16 ($n=3$), O163:H19 ($n=3$), ONT:H21 ($n=3$), O124:H19 ($n=2$), O8:H19 ($n=1$), O79:H28 ($n=1$), O91:H21 ($n=1$), O130:H11 ($n=1$), O174:H28 ($n=1$), ONT:HNT ($n=1$), and OR:H19 ($n=1$) serotypes, carried the *ehx*A and *saa* genes.

The clonal relatedness of 102 non-O157 STEC strains was established by *Xba*I-PFGE. In addition, 11 isolates were excluded from the analysis because of bacterial lyses. PFGE analysis showed 56 different patterns with 67.6% similarity, with 65 strains grouped in 19 clusters (I–XIX) of two to eight strains each and 100% similarity (Table 4). Unique patterns were observed for 37 strains. The cluster XIV grouped two O124:H19 strains isolated from rectal swabs and water in October 2005 and February 2006, respectively. We can speculate that the water was contaminated with manure, and the clone persisted in the environment for almost 5 months. Some

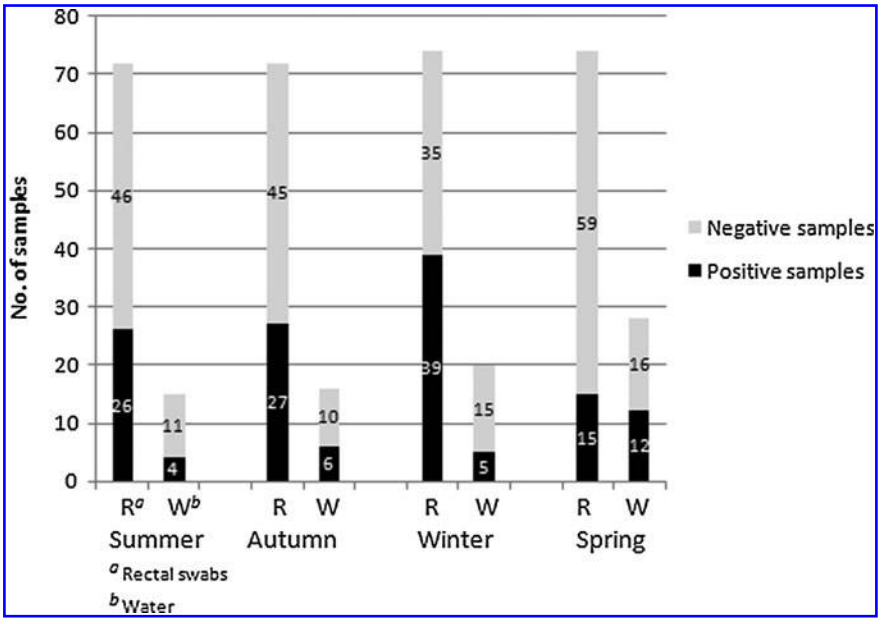

FIG. 1. Distribution of non-O157 Shiga toxin–producing *Escherichia coli* (STEC) polymerase chain reaction (PCR)–positive and PCR-negative samples per season, in a longitudinal study from a cattle farm in Argentina.

Table 2. Serotypes of Non-O157 Shiga Toxin–Producing *Escherichia coli* Strains Isolated from Bovine Rectums and Water Between September 2005 and November 2006, from a Cattle Farm in Argentina

Serotype	No. of isolates from: Cattle	No. of isolates from: Water	Total (%) of isolates
O103:[H2][a]	12		10.6
O136:H12	8		7.1
O178:H19	8		7.1
O103:NM[b]	5		4.4
O116:H21	4		3.5
O8:H16	3		2.7
O107:H7	3		2.7
O113:H21	3		2.7
O141:H49	3		2.7
O163:H19	3		2.7
O179:H8	3		2.7
O22:H8	2		1.8
O113:NM	2		1.8
O124:H19	1	1	1.8
O2:H25	1		0.9
O7:HNM	1		0.9
O8:H19	1		0.9
O15:H27	1		0.9
O22:H16	1		0.9
O79:H28	1		0.9
O84:H2	1		0.9
O91:H21	1		0.9
O130:H11	1		0.9
O174:H28	1		0.9
ONT:H2	17		15.0
ONT:H19	7		6.2
ONT:H21	7	1	7.1
ONT:HNT[c]	2	1	2.7
OR:H2	6		5.3
OR:H19	1		0.9

[a]The [H2] antigen was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).
[b]Nonmotile.
[c]Nontypeable.
Serotypes reported as human pathogens worldwide are shown in boldface, and serotypes reported as human pathogens in Argentina are underlined.

clones remained within the herd for several months, ONT:H21 strains (clusters III and XII) for 7 months; ONT:H2 (cluster II) and O178:H19 (cluster XIII) for 4 months; and ONT:H19 (cluster I) and O107:H7 (cluster XIX) for 3 months. Among the O178:H19 strains, three different clusters were detected (IV, V, and XIII). Strains O178:H19 from clusters IV and V showed 88.2% similarity with only four band differences, and 70% similarity with strains from cluster XIII.

Discussion

The prevalence of non-O157 STEC in cattle rectums was on average 36.6% (95% confidence interval, 31.3–42.3%). In previous studies from Argentina, Meichtri *et al.* (2004) found 69% STEC prevalence and Masana *et al.* (2011) reported a 22.3% prevalence of non-O157 STEC in feces of bovines. This variation could be attributed to differences in sampling procedures, age and weight of the animals, and feeding system.

Table 3. Virulence Genotypes of Non-O157 Shiga Toxin–Producing *Escherichia coli* Strains Isolated from Cattle Rectums and Water from a Farm in Argentina

Genotype	No. of isolates from: Cattle	No. of isolates from: Water	Total (%) of isolates
$stx_1/eae/ehxA$	19		16.8
$stx_2/saa/ehx$A	16	2	15.9
$stx_1/stx_2/saa/ehxA$	15	1	14.1
$stx_{2c(vh\text{-}a)}$	15		13.3
$stx_{2c(vh\text{-}b)}$	11		9.7
stx_1	10		8.8
$stx_{2c(vh\text{-}a)}/stx_{2c(vh\text{-}b)}$	6		5.3
$stx_2/stx_{2c(vh\text{-}b)}/saa/ehxA$	5		4.4
stx_2	2		1.8
$stx_2/stx_{2c(vh\text{-}a)}$	2		1.8
$stx_{2c(vh\text{-}b)}/saa/ehx$A	2		1.8
stx_{2NT}	2		1.8
stx_2/eae /ehxA	1		0.9
$stx_{2c(vh\text{-}a)}/eae/ehx$A	1		0.9
$stx_1/stx_{2c(vh\text{-}b)}/saa/ehxA$	1		0.9
$stx_1/stx_{2c(vh\text{-}b)}$	1		0.9
$stx_1/stx_2/stx_{2c(vh\text{-}b)}/saa/ehxA$	1		0.9

Major genotypes reported as human pathogens in Argentina are underlined.

In the present study, different types of animals bought by the farmer in fairs of Entre Ríos province were included. The herd grazed freely in open pasture during the day, and at night the animals received a grain supplementation. Once cattle reached a weight of at least 650 pounds (300 kg), they were transported to a local slaughterhouse.

The prevalence of non-O157 STEC in water was 34.2% (95% confidence interval, 24.4–45.1%). STEC O124:H19 strains with identical genotype and PFGE pattern were recovered from bovine and water sources. The sedimentary layers of watertroughs have been indicated as a possible environmental reservoir (Hancock *et al.*, 1998).

We found that 42.5% (48/113) of non-O157 STEC isolates belonged to serotypes associated with human disease worldwide, including Argentina. This frequency was lower than those reported by Masana *et al*. (2011) (53.6%) and Meichtri *et al*. (2004) (51.2%). The isolated strains could be sorted into four seropathotype groups described by Karmali *et al*. (2003). Seventeen (15%) STEC O103:[H2] and O103:NM strains belonged to seropathotype B. This widespread serotype has been associated with HUS cases in Argentina (Rivas *et al*., 2006) and has also been isolated from cattle and beef products (Blanco *et al*., 2004; Bettelheim *et al*., 2007; Masana *et al*., 2011). However, the serotypes O26:H11, O111:HNM, O121:H19, and O145:HNM, also included in seropathotype B, were not detected during the study.

Twenty-one (18.6%) STEC strains of O178:H19, O113:H21, O163:H19, O22:H8, O8:H19, O15:H27, O91:H21, O130:H11, and O174:H21 serotypes belonged to seropathotype C (Table 2). STEC O178:H19 strains were reported as the cause of an HUS case in Argentina (Giugno *et al*., 2007). STEC O113:H21 is an important serotype associated with HUS and hemorrhagic colitis in humans worldwide, including Argentina (Brett *et al*., 2003; Rivas *et al*., 2006). It was also recovered

Table 4. Distribution of Non-O157 Shiga Toxin–Producing *Escherichia coli* Strains in *Xba*I-PFGE Clusters, Serotype, Genotype, Number of Isolates, Origin, and Sampling Dates

Cluster	Xba*I-PFGE pattern*	*Serotype*	*Genotype*	*No. of isolates*	*Origin*	*Sampling date (no. of strains)*
I	AREXSX01.0442	ONT:H19	$stx_2/stx_{2c(vh\text{-}b)}/saa/ehxA$	5	B[a]	04/2006 (1), 05/2006 (2), 07/2006 (2)
II	AREXSX01.0426	ONT:H2	$stx_{2c(vh\text{-}a)}/stx_{2c(vh\text{-}b)}$	4	B	04/2006 (1), 05/2006 (1), 07/2006 (2)
III	AREXSX01.0440	ONT:H21	stx_2	3	B	01/2006 (1), 04/2006 (1), 07/2006 (1)
IV	AREXSX01.0324	O178:H19	$stx_{2c(vh\text{-}a)}$	2	B	04/2006 (1), 05/2006 (1)
V	AREXSX01.0326	O178:H19	$stx_{2c(vh\text{-}a)}$	2	B	04/2006 (1), 05/2006 (1)
VI	AREXSX01.0444	O141:H49	$stx_2/saa/ehxA$	3	B	07/2006(3)
VII	AREXWX01.0004	O103:H-	$stx_1/eae/ehxA$	9	B	07/2006 (9)
VIII	AREXSX01.0445	O179:H8	$stx_2/saa/ehxA$	2	B	07/2006 (2)
IX	AREXSX01.0437	ONT:H2	$stx_{2c(vh\text{-}a)}$	3	B	01/2006 (3)
X	AREXSX01.0423	ONT:H2	$stx_{2c(vh\text{-}b)}$	3	B	04/2006 (3)
XI	AREZJX01.0005	O22:H8	$stx_{2c(vh\text{-}b)}$	2	B	07/2006 (2)
XII	AREXSX01.0447	ONT:H21	$stx_1/stx_2/saa/ehxA$	2	B	01/2006 (1), 07/2006 (1)
XIII	AREXSX01.0348	O178:H19	$stx_1/stx_2/saa/ehxA$	4	B	01/2006 (1), 04/2006 (1), 05/2006 (1)
XIV	AREXSX01.0455	O124:H19	$stx_1/stx_2/saa/ehxA$	2	W[b],B	2/2006 (1), 10/2005 (1)
XV	AREXBX01.0023	O8:H16	$stx_1/stx_2/saa/ehxA$	2	B	07/2006 (2)
XVI	AREXSX01.0432	O116:H21	$stx_1/stx_2/saa/ehxA$	2	B	07/2006 (2)
XVII	AREXSX01.0435	O136:H12	stx_1	8	B	01/2006 (8)
XVIII	AREXWX01.0015	O103:H-	$stx_1/eae/ehxA$	5	B	04/2006 (4), 05/2006 (1)
XIX	AREXSX01.0456	O107:H7	$stx_{2c(vh\text{-}b)}$	2	B	10/2005 (1), 01/2006 (1)

[a]Bovine rectal swabs.
[b]Water.
PFGE, pulsed-field gel electrophoresis.

from ground beef (Bosilevac and Koohmaraie, 2011) and is one of the most frequent serotypes in Argentine cattle (Masana *et al*., 2011). STEC O22:H8 and O91:H21 strains have been isolated from HUS cases (Blanco *et al*., 2004; Lindgren, 1993). The frequency of STEC O8:H19 (0.9%) found in the present study was lower than that reported by Meichtri *et al.* (2004) (12.9%) and Masana *et al.* (2011) (7.9%). Isolates of serotypes O8:H16, O179:H8, O113:NM, O22:H16, and O84:H2 that occasionally produce a mild disease (seropathotype D) represented 8.8% (10/113) of the isolates. Non-O157 STEC strains included in seropathotype E represented 57.5% of the isolates.

The ability of STEC strains to produce bloody diarrhea and HUS in humans has been associated mainly with the expression of stx_2 gene variants, especially in combination with the *eae* gene (Böerlin *et al*., 1999). Similarly to other studies conducted in the Argentine bovine reservoir, most strains were stx_2^+ (74.3%) alone or combined with stx_1. The four most frequent genotypes—$stx_1/eae/ehxA$; $stx_2/saa/ehxA$; $stx_1/stx_2/saa/ehxA$; and $stx_{2c(vh\text{-}a)}$—were detected with incidence rates of 16.8% to 13.3%. Forty-three (38%) non-O157 STEC strains carried the *saa* gene, but only 21 (18.6%) harbored the *eae* gene. The *ehx*A gene was present in 64 (56.6%) strains, associated with *saa* (38%) or *eae* (18.6%) genes. The $stx_1/eae/ehxA$ genotype was harbored by 12 strains of O103:[H2], five of O103:NM, and two of ONT:H21 serotypes. The $stx_2/eae/ehxA$ and $stx_{2c(vh\text{-}a)}/eae/ehxA$ genotypes were harbored by two nontypeable strains.

When the *Xba*I-PFGE patterns were compared with those included in the Argentine PulseNet Database, the following matches were identified: two O22:H8/$stx_{2c(vh\text{-}b)}$ strains included in cluster XI showed an identical AREZJX01.0005 pattern to one strain isolated from an HUS case (2008) and two strains isolated from hamburgers (2008). Two O178:H19/$stx_{2c(vh\text{-}b)}$ strains of cluster IV showed an identical AREXSX01.0324 pattern to one strain isolated from ground beef in 2006, and another two strains of cluster V had an identical AREXSX01.0326 pattern with one strain isolated from ground beef in 2007. Some strains characterized in this study showed identical *Xba*I-PFGE profiles with strains isolated from bovines in other Argentine provinces (Masana *et al*., 2011). Only two strains with identical serotype, genotype, and PFGE profile were recovered from rectal swabs in October 2005 and water in February 2006, which indicates bovine manure as a source of STEC contamination to environmental water. On the other hand, the identification of identical clones isolated in different periods of time highlights the importance of the horizontal fecal-oral transmission of these pathogens among cattle.

The prevalence of non-O157 STEC in bovine rectums during the present survey, with a higher frequency in winter (52.7%), $p < 0.05$, did not show the expected distribution along the seasons (Fig. 1). Heavy rains (240.5 mm) and unusual temperature (10.6–19.8°C) in May, June, and July (the winter) might have contributed to the great pollution of the water (Boletín Climatológico, 2006).

Conclusion

By PCR screening, a similar prevalence of non-O157 STEC was found in cattle rectums (36.6%) and water (34.2%).

However, the strain recovery from water samples was lower than those from animal sources. Culture-based methods in this kind of water samples need to be improved. By comparison, some of the strains found in this study showed *Xba*I-PFGE patterns indistinguishable from those isolated from HUS cases, and meat and animal samples included in the National PulseNet Database. Two O124:H19 strains of stx_1/stx_2/*saa*/*ehxA* genotype with indistinguishable profile were isolated from rectal swabs and water with a 5-month interval. The results of the present study reinforce the assumption of manure as a source of contamination and the need to establish preharvest control strategies to reduce the transmission of these group of pathogens among cattle and also to food crops, water, and the environment.

Disclosure Statement

No competing financial interests exist.

References

Bettelheim KA. The non-O157 Shiga-toxigenic (Verocytotoxigenic) *Escherichia coli*; under-rated pathogens. Crit Rev Microbiol 2007;33:67–87.

Beutin L, Montenegro MA, Orskov I, Orskov F, Prada J, Zimmermann S, Stephan R. Close association of Verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J Clin Microbiol 1989;27:2559–2564.

Blanco M, Blanco JE, Mora A, Dahbi G, Alonso MP, González EA, Bernárdez MI, Blanco1 J. Serotypes, virulence genes, and intimin types of Shiga toxin (Verotoxin)–producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (*eae-ξ*). J Clin Microbiol 2004;42:645–651.

Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Association between virulence factors of Shiga toxin–producing *Escherichia coli* and disease in humans. J Clin Microbiol 1999;37:497–503.

Boletín Climatológico, July 2006. Vol. XVIII, No. 7. Argentine Air Force, Argentine National Weather Service.

Bosilevac JM, Koohmaraie M. Prevalence and characterization of non-O157 Shiga toxin–producing *Escherichia coli* isolated from commercial ground beef in the United States. Appl Environ Microbiol 2011;77:2103–2112.

Brett KN, Ramachandran V, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP. stx_{1c} is the most common Shiga toxin 1 subtype among Shiga toxin–producing *Escherichia coli* isolates from sheep but not among isolates from cattle. J Clin Microbiol 2003;41:926–936.

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 2005;192:1422–1429.

Ewing WH, Edwards PR. *Edwards and Ewing's Identification of Enterobacteriaceae.* New York: Elsevier, 1986.

Giugno SM, Bibiloni N, Rahman R, *et al.* Association between uremic hemolytic syndrome and infection by Shiga toxin–producing *Escherichia coli*. Acta Bioquím Clín Latinoam 2007;41:27–33.

Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE, Carpenter LV. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA. Prev Vet Med 1998;35:11–19.

Karch H, Böhm H, Schmidt H, Gunzer F, Aleksic S, Heesemann J. Clonal structure and pathogenicity of Shiga-like toxin–producing, sorbitol-fermenting *Escherichia coli* O157:H7. J Clin Microbiol 1993;31:1200–1205.

Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K, Kaper JB. Association of genomic O island 122 of *Escherichia coli* EDL933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. J Clin Microbiol 2003;41:4930–4940.

Leotta GA, Chinen I, Epszteyn S, Miliwebsky E, Melamed IC, Motter M, Ferrer M, Marey E, Rivas M. Validación de una técnica de PCR múltiple para la detección de *Escherichia coli* productor de toxina Shiga. Rev Arg Microbiol 2005;37:1–11. (In Spanish.)

Lindgren SW, Melton AR, O'Brien AD. Virulence of enterohemorrhagic *Escherichia coli* O91:H21 clinical isolates in an orally infected mouse model. Infect Immun 1993;61:3832–3842.

Machado J, Grimont F, Grimont PAD. Identification of *Escherichia coli* flagellar types by restriction of the amplified *fli*C gene. Res Microbiol 2000;151:535–546.

Mathusa EC, Chen Y, Enache E, Hontz L. Non-O157 Shiga toxin–producing *Escherichia coli* in foods. J Food Prot 2010;73:1721–1736.

Meichtri L, Miliwebsky E, Gioffré A, Chinen I, Baschkier A, Chillemi G, Guth BE, Masana MO, Cataldi A, Rodríguez HR, Rivas M. Shiga toxin–producing *Escherichia coli* in healthy young beef steers from Argentina: Prevalence and virulence properties. Int J Food Microbiol 2004;96:189–198.

Masana MO, D'Astek BA, Palladino PM, Galli L, Del Castillo LL, Carbonari C, Leotta GA, Vilacoba E, Irino K, Rivas M. Genotypic characterization of non-O157 Shiga toxin–producing *Escherichia coli* in beef abattoirs of Argentina. J Food Prot 2011;74:2008–2017.

Piérard D, Muyldermans G, Moriau L, Stevens D, Lauwers S. Identification of new verocytotoxin type 2 variant B-subunit genes in human and animal *Escherichia coli* isolates. J Clin Microbiol 1998;36:3317–3322.

Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 2006;3:59–67.

Rivas M, Miliwebsky E, Chinen I, *et al.* Epidemiología del síndrome urémico hemolítico en Argentina. Diagnóstico del agente etiológico, reservorios y vías de transmisión. Medicina (Buenos Aires) 2006;66:27–32. (In Spanish.)

Rivas M, Chinen I, Miliwebsky E, *et al.* Epidemiology of Argentinean Shiga toxin–producing *Escherichia coli.* In: *Population Genetics of Bacteria: A Tribute to Thomas S. Whittam.* Walk ST, Feng PCH (eds.). Whashington, DC: ASM Press, 2011, pp. 109–132.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—Major pathogens. Emerg Infect Dis 2011;17:7–15.

Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect Immun 1995;63:1055–1061.

Smith HR, Willshaw GA, Cheasty T, *et al.* Verocytotoxin-producing *Escherichia coli* in England and Wales. In: *Conference Proceedings on Epidemiology of Verocytotoxigenic* E. coli. *Organised by an EU Concerted Action on Verocytotoxigenic* E. coli *(CT98-3935).* Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA (eds.). Malahide, Dublin, Ireland, 2001, pp. 28–34.

Spizzirri FD, Rahman RC, Bibiloni N, Ruscasso JD, Amoreo OR. Childhood hemolytic uremic syndrome in Argentina: Long-

term follow-up and prognostic features. Pediatr Nephrol 1997;11:156–160.

Tanaro JD, Leotta GA, Lound LH, Galli L, Piaggio MC, Carbonari CC, Araujo S, Rivas M. *Escherichia coli* O157 in bovine feces and surface water streams in a beef cattle farm of Argentina. Foodborne Pathog Dis 2010;7:475–477.

Tyler SD, Johnson WM, Lior H, Wang G, Rozee KR. Identification of Verotoxin type 2 variant B subunit genes in *Escherichia coli* by the polymerase chain reaction and restriction fragment length polymorphism analysis. J Clin Microbiol 1991;29:1339–1343.

Zhang W, Bielaszewska M, Kuczius T, Karch H. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (stx(1c)) in *Escherichia coli* strains isolated from humans. J Clin Microbiol 2002;40:1441–1446.

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