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Profile of Shiga toxin-producing *Escherichia coli* strains isolated from dogs and cats and genetic relationships with isolates from cattle, meat and humans

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ABSTRACT

Pets can be reservoirs of Shiga toxin-producing *Escherichia coli* (STEC) strains. The aim of this study was to examine nine strains belonging to several serotypes (O91:H21, O91:H16, O178:H19, O8:H19, O22:H8, O22:HNT, ONT:H8), previously recovered from cats or dogs. To this end, we assessed a set of additional virulence genes (stx_2 subtype, *subAB*, *ehxA*, *eae* and *saa*), cytotoxic activity, and genetic relationships with strains isolated from cattle, meat and humans using pulsed-field gel electrophoresis (PFGE). Most of the isolates carried the stx_2 and/or $stx_{2vh\text{-}b}$ sequences, while only the O91:H21 isolate presented the mucus-activatable stx_{2d} variant, as confirmed by sequencing the genes of subunits A and B. All the strains showed cytotoxic activity in cultured cells. One of the two O178:H19, selected for its high level of cytotoxicity in Vero cells, showed the ability to cause functional alterations in the human colon mucosa *in vitro*. None of the strains possessed the *subAB*, *eae* or *saa* genes and only the strains belonging to serotype O8:H19 carried the *ehxA* gene. The isolates shared 90–100% similarity by PFGE to epidemiologically unrelated strains of the corresponding serotypes recovered from cattle, meat or humans. Our results demonstrate that dogs and cats may have a role in the infection of humans by STEC, probably serving as a vehicle for bovine strains in the cycle of human infection, and thus emphasize the health risks for owners and their families.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is a food-borne pathogen that causes diarrheal diseases, sometimes followed by hemolytic uremic syndrome (HUS), a systemic complication that could lead to death. Cattle and other ruminants have been pointed out as major reservoirs for STEC (Meichtri et al., 2004; Mercado et al., 2004). Although most outbreaks and sporadic cases of HUS worldwide are attributed to *E. coli* O157:H7, it is known that a large number of cases of severe illness are caused by other serotypes (Beutin et al., 1998; Rivas et al., 2006).

STEC strains produce two potent cytotoxins (Stx1, Stx2), which inhibit protein synthesis. A growing number of Stx1 and Stx2 variants have been identified based on the amino acid composition of their StxA and StxB subunits. Production of Stx2 and their variants Stx2c ($stx_{2vh\text{-}a}$ and $stx_{2vh\text{-}b}$) as well as of mucus-activatable Stx2d toxins has

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been found to be closely associated with HUS and bloody diarrhea in humans (Schmitt et al., 1991; Beutin et al., 2007). Another virulence factor of highly virulent serotypes in humans is the outer membrane protein intimin, which is encoded by the *eae* gene on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE) (McDaniel et al., 1995). This locus is responsible for attaching and effacing lesions in the intestinal mucosa. In addition, STEC strains associated with severe disease in humans produce an enterohemolysin (EHEC hemolysin) encoded by the plasmid-borne gene *ehx*A (Schmidt et al., 1995). The subtilase cytotoxin, a new member of the AB_5 toxin family encoded by the *subAB* operon (Paton et al., 2004), and the auto-agglutinating adhesin designated Saa (STEC autoagglutinating adhesin) have been detected among some human pathogenic serotypes devoid of *eae* (Paton et al., 2001).

Although domestic pets shed STEC belonging to diverse serotypes in their feces (Bentancor et al., 2007; Staats et al., 2003), there is a lack of information on their virulence properties and their genetic relationship with STEC strains of human origin. The objective of this study was to further characterize a set of STEC strains isolated from dogs and cats in order to assess their pathogenic potential and establish their relationship with human and bovine strains isolated in the same country, Argentina, throughout the last years.

2. Materials and methods

2.1. Bacterial strains

A total of nine STEC strains isolated from dogs and cats as part of an epidemiological survey carried out in Buenos Aires, Argentina (Bentancor et al., 2007) were studied. These strains were initially characterized as belonging to the following serotypes: O8:H19 (formerly identified as ONT:H19, $n = 2$), O22:HNT ($n = 1$), O22:H8 ($n = 1$), O91:H16 ($n = 1$), O91:H21 ($n = 1$), O178:H19 ($n = 2$), and ONT:H8 ($n = 1$).

2.2. Detection of STEC virulence genes

All strains were retested by PCR for virulence determinants stx_1, stx_2 and *eae* for comparison with previous results (Pollard et al., 1990; Bentancor et al., 2007). The STEC strains were also analyzed for the presence of the *subAB* (subtilase cytotoxin) (Paton and Paton, 2005), *ehxA* (EHEC hemolysin) and *saa* (STEC autoagglutinating adhesin) genes, as described elsewhere (Schmidt et al., 1995; Paton et al., 2001).

2.3. stx_2 subtyping

The stx_2 gene was subtyped by PCR-RFLP analysis of the B subunit gene to discriminate between the stx_2, $stx_{2vh\text{-}a}$ and $stx_{2vh\text{-}b}$ variants (Tyler et al., 1991). Restriction patterns were obtained after digestion of the amplified products by *Hae*III, *Rsa*I, and *Nci*I (Promega, WI, USA). The variant genes $stx_{2\text{-}O118}$ (formerly $stx_{2d\text{-}Ount}$) and $stx_{2\text{-}OX3}$ (formerly $stx_{2d\text{-}OX3}$) were investigated using PCR with primers VT2cm-VT2f, as described by Pierard et al. (1998). All the isolates were tested for the presence of the mucus-activatable stx_2 genes (stx_{2d}) by restriction analysis, using the *Pst*I site of the SLT-II-vc/CKS2 fragment, which amplifies part of the subunit A and all the subunit B genes (Jelacic et al., 2003). Absence of the *Pst*I site is used as an indicator of the presence of a putative mucus-activatable stx_{2d} variant. Strains identified as stx_{2d} at screening were then analyzed by the one-step PCR method described by Zheng et al. (2008). In order to confirm the presence of the mucus-activatable stx_{2d} variant, we sequenced the 890 bp amplicon generated by the SLT-II-vc/CKS2 primer pair using a *Pfx*DNA polymerase (Qiagen, USA). PCR products were purified (Wizard SV Gel and PCR Clean up System, Promega) and sequenced (ABI PRISM 3130XL genetic analyzer, Applied Biosystems). Nucleotide sequence alignments were performed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The identities of any two sequences were compared using the BLAST 2 SEQUENCES program (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html). Predicted amino acid alignments were prepared with the Multalin Sequence Alignment program (http://multalin.toulouse.inra.fr/multalin/). The following sequences were used as reference: stx_2 (phage 933W, GenBank accession no. AF125520) and stx_{2d} (*E. coli* O91:H21 strain B2F1, GenBank accession no. AF479829).

2.4. Pulsed-field gel electrophoresis (PFGE)

The macrorestriction fragment separation by PFGE was performed using the 24-h PulseNet standardized PFGE protocol for *E. coli* O157:H7, with minor modifications (CDC, 2007). Digestion was carried out with 25 U of *Xba*I (Promega) for 18 h at 37 °C. *Bln*I (*Xmaj*, Fermentas/50 U for 18 h at 37 °C) was used as second enzyme to confirm identical *Xba*I-PFGE patterns. The standard strain *Salmonella* Braenderup H9812 was gently provided by CDC. DNA fragments were resolved in 1% agarose gel in 0.5× Tris borate EDTA electrophoresis buffer at 14 °C, in a contour clamped homogeneous electric field (CHEF) DR-III electrophoresis chamber (Bio-Rad Laboratories, Hercules, CA). The run time was 18 h, with a constant voltage of 200 V, using a linear pulse ramp of 2.2–54.2 s. The TIFF images obtained by PFGE were analyzed with the BioNumerics version 4.6 software package (Applied Maths, Belgium). The relatedness among the patterns was estimated by the proportions of shared bands, after applying the Dice coefficient. The UPGMA method was used to generate dendrograms with 1.5% tolerance values. The analysis of the patterns was confirmed visually. The pattern designation used is that recommended by the CDC for PulseNet International. The *Xba*I-PFGE patterns were compared with those obtained from strains of the same serotype isolated between 1988 and 2009 from different sources included in the Argentine *E. coli* O157 (n: 1865) and non-O157 STEC (n: 1217) Database (DB), which consisted of the 57 O8:H19, 52 O178:H19, 21 O91, and 11 O22 isolates.

2.5. Preparation of Stx2 and dosage of the cytotoxic activity on Vero cells

Strains were incubated overnight at 37 °C with shaking at 200 rpm in 5 ml of Luria–Bertani broth (Difco

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Laboratories, USA). Bacterial cells were then removed by centrifugation, and the resultant supernatant (sStx2) was filtered through 0.22 μm pore size filter units (Millipore Corp., USA) and assayed for toxicity to Vero cells as previously described (Fiorito et al., 2000). The 50% cytotoxic dose (CD_{50}) corresponded to the dilution required to kill 50% of Vero cells. Supernatant from *E. coli* DH5α was included as a negative control. The results are the mean of three experiments.

2.6. Measurement of transepithelial net water flux in the Ussing chamber

One strain with high levels of cytotoxicity in Vero cells was selected to measure its effect on absorptive water flow (Jw) in human colon using the Ussing chamber. Colon fragments were removed from surgical ablations obtained from adult cancer patients with their consent and the approval of the Ethics Committee of the School of Medicine of the University of Buenos Aires, Argentina. Immediately after ablation, tissues from the macroscopically healthy area were taken to the laboratory in a high-potassium Ringer solution at 4 °C to preserve transport functions. The colonic mucosa was then separated from the rest of the tissue and mounted as a diaphragm in a modified Ussing chamber with a 1.76 cm^2 area connected to a special electro-optical device (Fiorito et al., 2000). Briefly, the tissue was bathed with a standard Ringer solution (113 mM NaCl, 4.5 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1.2 mM K_2HPO_4, 0.2 mM KH_2PO_4, 25 mM glucose) at 37 °C, bubbled with a 95% O_2/5% CO_2 gaseous mixture to maintain normal physiological conditions and held against a nylon mesh by a hydrostatic pressure of 10 cm of water. Transepithelial water flux (Jw, μl/min cm^2) across the colonic mucosa was measured by displacement of a photo-opaque solution inside a glass capillary tube connected to the mucosal side of the chamber via an intermediate chamber. The movement of the liquid meniscus in the glass capillary was detected using the electro-optical device connected to a computer. The sensitivity of the instrument is ±0.05 μl. When the basal Jw was stabilized, 200 μl of an overnight whole culture of *E. coli* #276 (O178:H19) was added to the mucosal side of the tissue (time 0) and Jw was recorded for 60 min. *E. coli* K12 DH5α whole culture was used as a negative control. Each assay was carried out three times. Data are expressed as ΔJw, where ΔJw = Jw at a given time – Jw at time 0. Results are expressed as means ± SEM. Statistical significance between two mean values were determined using the Student's *t*-test. To evaluate the changes of Jw as a function of time, all data from the curves were analyzed by a *t*-test for independent samples and the statistical significance was $p \leq 0.05$.

3. Results

A summary of the characteristics of the STEC strains identified in this study is shown in Table 1 Table 1. None of the strains possessed the *subAB*, *eae* or *saa* genes and only the strains belonging to serotype O8:H19 carried the *ehxA* gene. According to the PCR-RFLP analysis of the B subunit gene, the O8:H19, O22:H8 and O91:H16 isolates contained the stx_2 gene, while the O91:H21 and O178:H19 isolates contained the $stx_{2vh\text{-}b}$ variant. The O22:HNT and ONT:H8 isolates contained both the stx_2 and $stx_{2vh\text{-}b}$ sequences. Although serotypes O8:H19, O91:H16, O91:H21 and O178:H19 were positive for the stx_{2d} variant by PCR and RFLP analyses, only the O91:H21 isolate was confirmed by sequencing as carrying the respective amino acid substitutions in the A and B subunits described for mucus-activatable toxins (Fig. 1). All the strains showed cytotoxic activity on Vero cells. The level of cytotoxicity of culture supernatants varied from 10^2 to 10^5 CD_{50}/ml (Table 1). These cytotoxic levels were in the same order as or above the one found in an *E. coli* O157:H7 strain (10^3 CD_{50}/ml) isolated from human HUS in Argentina (Fiorito et al., 2000). *E. coli* DH5α used as negative control did not cause significant cytotoxicity on Vero cells (<10 CD_{50}/ml).

We then investigated the ability of strain #276 (O178:H19), which showed high levels of cytotoxicity in Vero cells, to cause physiological changes in the human gut. A reduction of Jw (Fig. 2) was observed following the addition of this strain to the mucosal side of a human colon tissue sample in the Ussing chamber. This reduction was already significant after 10 min of incubation ($p < 0.01$).

The PFGE analysis produced 15–24 fragments ranging in size from approximately 20 to 600 kb. A comparative

Table 1
Characteristics of Shiga toxin-producing *Escherichia coli* strains from dogs and cats analyzed in this study.

Strain	Origin	Serotype	stx_2[a]	stx_{2d} genotype		CD_{50}/ml[b]	*ehxA*	*subAB*	*eae*	*saa*
				PCR[c]	Sequencing[d]					
235IV	Dog	O91:H16	stx_2	P	A	5.0	–	–	–	–
248	Dog	O91:H21	$stx_{2vh\text{-}b}$	P	P	2.0	–	–	–	–
303	Cat	O8:H19	stx_2	P	A	4.0	+	–	–	–
330	Cat	O8:H19	stx_2	P	A	4.7	+	–	–	–
276	Dog	O178:H19	$stx_{2vh\text{-}b}$	P	A	4.7	–	–	–	–
377iii	Dog	O178:H19	$stx_{2vh\text{-}b}$	A	A	4.7	–	–	–	–
394	Cat	O22:H8	stx_2	A	ND[e]	4.7	–	–	–	–
422.c	Cat	O22:HNT	$stx_2 + stx_{2vh\text{-}b}$	A	ND[e]	4.0	–	–	–	–
422.p	Cat	ONT:H8	$stx_2 + stx_{2vh\text{-}b}$	A	ND[e]	5.0	–	–	–	–

[a] Results obtained by PCR and RFLP analyses according to Tyler et al. (1991).
[b] Results CD_{50}/ml expressed as log 1/dilution.
[c] Results obtained using PCR-.based analysis (Jelacic et al., 2003; Zheng et al., 2008). P: presence of the stx_{2d} genotype, A: absence of the stx_{2d} genotype.
[d] Results obtained by sequencing and database analysis.
[e] ND, not determined.

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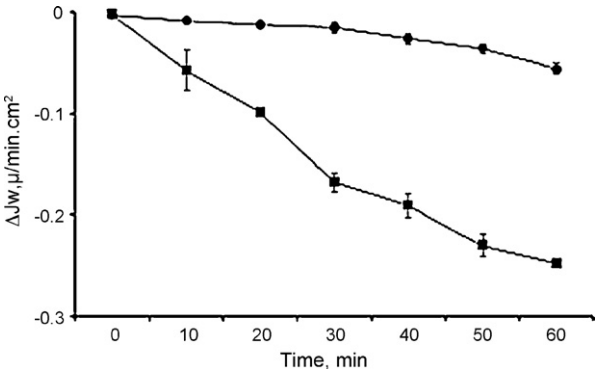

Fig. 1. Measurement of transepithelial net water flux in the Ussing chamber. Time course of the transepithelial net water flux change (ΔJw) measured in human colon after the addition of the *E. coli* #276 (O178:H19) strain (■). The *E. coli* K12 DH5α strain was used as a negative control (●). Error bars represent the standard errors from the mean of three experiments. Strain #276 showed an inhibition of Jw depending on the incubation time and became statistically significant 10 min after the exposure to the strain ($p < 0.01$), continuing to decrease throughout the 60-min observation period.

dendrogram obtained from the analysis of the PFGE patterns (Fig. 3) revealed that the isolates shared 90–100% of the bands with epidemiologically unrelated strains of the same serotypes recovered from cattle, meat and humans. The two O178:H19 isolates shared 91.4% of similarity in the *Xba*I-PFGE profiles, with differences in only three bands (Fig. 3A). One of them (strain #276) showed 97% of similarity with a strain recovered from beef cattle in 2006. The other O178:H19 strain (#377), isolated from a dog, yielded a *Xba*I-PFGE pattern identical to that of a strain recovered from a steer in 2007 and showed a high similarity (95%) by *Bln*I-PFGE, with only one band of difference. The *Xba*I-PFGE pattern of strain #248, isolated from a dog, was identical to that of a STEC O91:H21 isolated from a case of bloody diarrhea occurred in 2001 (*Bln*I-PFGE not done), whereas that of the STEC O91:H16 strain #235IV showed 91.5% of similarity with the strain isolated from a steer in 2006 (Fig. 3B). The two O8:H19 strains shared at least 91.4% of the *Xba*I-PFGE pattern with the most related O8:H19 strains included in the database, whereas one of them (#303) (Fig. 3C) showed *Xba*I- and *Bln*I-PFGE patterns indistinguishable from those yielded by a strain isolated from ground beef in 2007. The O22:H8 strain #394 showed an indistinguishable pattern by *Xba*I-PFGE and only one band of difference by *Bln*I-PFGE from those corresponding to the strains recovered from hamburger and bovine meat, in 2004 and 2007, respectively (Fig. 3D). Moreover, the O22:HNT strain shared 95% similarity of the PFGE profile with a strain isolated from a steer in 2007.

4. Discussion

We have previously demonstrated the presence of STEC in the intestinal content of dogs and cats from Buenos Aires

A

307

LNRKSQFLYTTGK	Stx2
LNRKSQ**S**LYTTG**E**	Stx2d2
LNRKSQ**S**LYTTG**E**	248
LNRKSQ**S**LYTTG**E**	235iv
LNRKSQFLYTTGK	377iii
LNRKSQ**S**LYTTG**E**	303
LNRKSQ**S**LYTTG**E**	330
LNRKSQFLYTTGK	276

B

1

Stx2	MKKMFMAVLFALASVNA MAADCAKGKIEFSKYNED
Stx2d2	MKKMFMAVLFALASVNA MAADCAKGKIEFSKYNE**N**
248	MKKMFMAVLFALVSVNA MAADCAKGKIEFSKYNE**N**
235iv	MKKMFMAVLFALVSVNA MAADCAKGKIEFSKYNED
377iii	MKKMFMAVLFALASVNA MAADCAKGKIEFSKYNE**N**
303	MKKMFMAVLFALVSVNA MAADCAKGKIEFSKYNED
330	MKKMFMAVLFALVSVNA MAADCAKGKIEFSKYNED
276	MKKMFMAVLFALVSVNA MAADCAKGKIEFSKYNE**N**

36

DTFTVKVDGKEYWTSR	Stx2
DTFTVKV**A**GKEYWTSR	Stx2d2
DTFTVKV**A**GKEYWTSR	248
DTFTVKVDGKEYWTSR	235iv
DTFTVKV**A**GKEYWTSR	377iii
DTFTVKVDGKEYWTSR	303
DTFTVKVDGKEYWTSR	330
DTFTVKV**A**GKEYWTSR	276

Fig. 2. Stx2 A and B subunit amino acid alignment. Comparison of C-terminal ends of the A and B subunits of six isolates carrying mucus-activatable stx_{2d} by PCR-based methods. Amino acids in bold and underlined are indicative of mucus activatable signature. The amino acid sequence corresponding to the mucus-activatable stx_{2d} variant is shaded.

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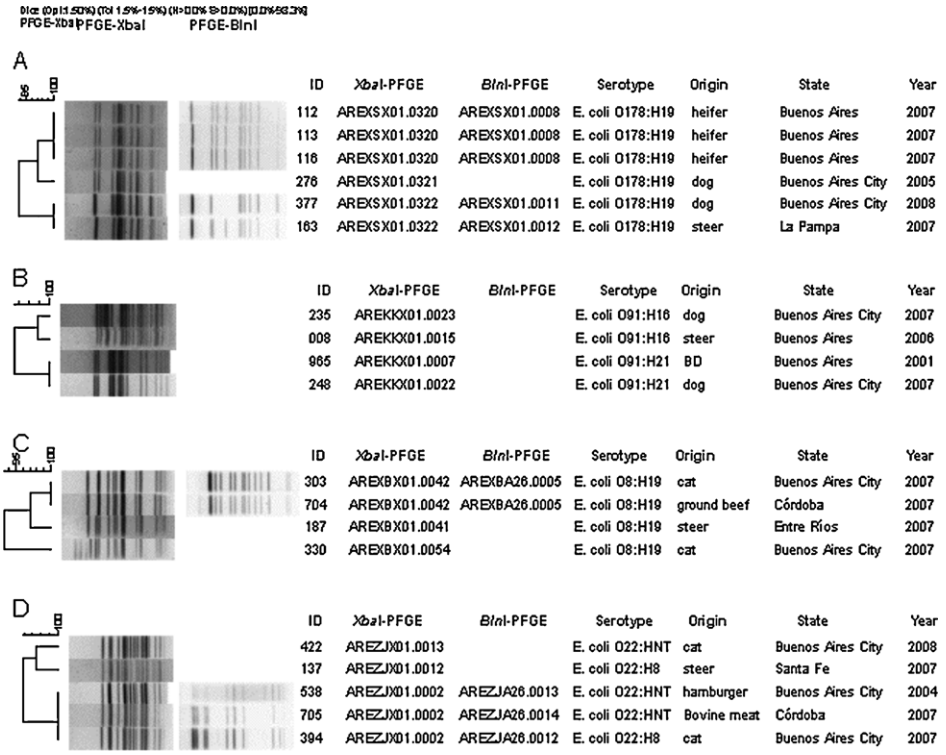

Fig. 3. *Xba*I-PFGE dendrograms of Shiga toxin-producing *Escherichia coli* strains including isolates from the Argentine Pulse Net Database. The dendrograms were produced by the UPGMA algorithm based on a Dice similarity coefficient with a 1.5% band position tolerance.

city and its surroundings (Bentancor et al., 2007), an area with high incidence of HUS in children. In this study, we further characterized the isolates for a set of additional virulence genes (*stx*$_2$ subtype, *subAB*, *ehxA*, *eae* and *saa*) and cytotoxic activity, and searched for genetic relationships with STEC strains isolated from cattle, meat and humans in Argentina in the last years, based on their PFGE profiles.

Most of the strains presented the *stx*$_2$ or/and *stx*$_{2vh\text{-}b}$ variants. These results are similar to previous reports concerning the distribution of *stx* genes in STEC isolated from cattle (Brett et al., 2003). The *stx*$_2$ is strongly associated with an increased risk of HUS (Rivas et al., 2006) and has been found to be the most prevalent *stx* gene in non-O157 isolates from cattle and beef products in Argentina (Blanco et al., 2004). In the present work, the PCR methods assayed for detection of the *stx*$_{2d}$ variant were complemented by sequence analysis of the genes of the A and B subunits, confirming the presence the *stx*$_{2d}$ variant only in the O91:H21 isolate (Melton-Celsa et al., 2002; Zheng et al., 2008).

Expression of the *stx* genes regulates the amount of Stx produced during infection, a critical feature of STEC pathogenicity correlated with the severity of human infection (Baker et al., 2007). In the present work, all strains except those of O91:H21 serotype produced high levels of Shiga toxins *in vitro*. The inhibition of the net absorption of water through the human colon by strain #276 (O178:H19) was indicative of its pathogenicity for humans. STEC O178:H19 are frequently isolated from cattle in Argentina (Blanco et al., 2004). To our knowledge, this is the first study showing that an *E. coli* strain isolated from pets is capable of causing functional alterations in the human colon *in vitro*. The low CD_{50} in Vero cells of the O91:H21 isolate, which carried the *stx*$_{2d}$ variant, probably correlates with the fact that it was measured in the absence of mouse intestinal mucus or elastase. The Stx2-activatable toxin was first described in the strain B2F1, an O91:H21 strain with two toxin alleles, Stx2d1 and Stx2d2 (formerly designated vh-a and vh-b by Teel et al., 2002). This toxin can be rendered more active by treatment with elastase present in the intestinal mucus, which cleaves two amino acids from the C-terminus of the Stx2d A2 peptide. We used the designation Stx2d for the mucus-activatable Stx2 type, as previously suggested (Beutin et al., 2007). The two types of mucus-activatable genes, called *stx*$_{2d1}$ and *stx*$_{2d2}$, were described firstly as subsets of the toxin B subunits *stx*$_{2v\text{-}ha}$ and *stx*$_{2v\text{-}hb}$ in the toxin B subunit, respectively, but the association of the mucus-activatable *stx*$_{2d}$ type with B-subunit variants is not absolute (Gobius et al., 2003). Therefore, in this work, we used the term *stx*$_{2d}$ to designate all types of mucus-activatable *stx*$_2$ genes.

Most of the STEC serotypes characterized in the present study were previously recovered from cattle and/or associated with cases of severe illness in humans. The O91:H21 serotype was isolated from cattle, hamburgers and ground beef (Madic et al., 2009; Meichtri et al., 2004), and, importantly, associated with cases of HUS in several countries, including Argentina (Ito et al., 1990; Giugno et al., 2007). STEC serotype O91:H16 was isolated from sheep in Europe (Zweifel et al., 2004). Serotype O178:H19 was

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detected in healthy cattle and meat (Blanco et al., 2004), but only recently associated with human disease, when it was isolated in Brazil from children suffering from diarrhea (De Toni et al., 2009) and in Argentina from a pediatric patient with HUS (Giugno et al., 2007).

The two O8:H19 strains isolated from cats carried the stx_2 gene and showed high cytotoxic activity in Vero cells. Although these strains were classified as carriers of the stx_{2d} variant according to the Jelacic et al. (2003) and Zheng et al. (2008) protocols, sequencing of the stx_2 genes showed that they did not fully correspond to mucus-activatable variants. Similar findings were previously reported by Tasara et al. (2008). However, both O8:H19 strains carried the *ehxA* gene and expressed the enterohemolytic phenotype. Strains of this serotype were isolated from three clinical cases of HUS in Argentina, but did not match by PFGE with the strains isolated from pets in this study (Argentinean Data Base of non-O157 STEC, unpublished data).

The O22 strains were isolated from cats and corresponded to serotypes previously found in clinical isolates from humans in Germany (Rüssmann et al., 1995) and cattle. STEC O22:H8 were predominant in cattle from Brazil (Timm et al., 2007) as well as among the serotypes most frequently isolated from cattle in France (Pradel et al., 2000). In Argentina, *E. coli* O22:H8 was isolated from cattle (Blanco et al., 2004) food and one HUS case (Argentinean Data Base of non-O157 STEC, unpublished data).

Since PFGE has a high discriminatory power to differentiate between epidemiologically unrelated strains of the same serotype, it has been used for investigations of several outbreaks of *E. coli* O157 infections (Barret et al., 1994). In the present work PFGE analysis revealed that most of the strains were closely related to strains of the same serotype isolated from cattle, meat or humans in the same or in distant geographical areas, suggesting a genomic relationship. However, there was no epidemiological information to support these relationships. Sporadic PFGE matches or clusters may be detected by molecular epidemiology, but the surveillance system is not sensitive enough to determine the epidemiological link.

In the present work, we studied STEC strains obtained from pets living in urban areas, often fed included raw beef, according with their owner habits (Bentancor et al., 2008). There are few references to the risk of zoonotic transmission by pets. Sporadic cases of HUS could be caused by STEC transmission from a household pet to humans, but the carrier rate of these strains by pets is probably low. Kataoka et al. (2010) found that dogs and cats, as companion animals, rarely harbor O157:H7 strains in their intestinal content. Moreover, if an owner was infected by STEC, the transmission from owner to pet should be considered. According to the close relationship in the cities between pets and their owners, transmission in both directions may thus take place.

5. Conclusion

Some of the STEC strains characterized in our study (O8:H19, O22:H8, O91:H21, O178:H19) belonged to pathotypes that have been associated with severe human disease, including HUS. The virulence profile of these strains suggests that their source might be the bovine meat used by owners to feed their pets. Therefore, there is a growing concern about the risk for human health associated with the presence of STEC strains with high expression level of the stx_2 variants in domestic pets. The evidence presented here highlights the epidemiological role of pets as a source of STEC human infections.

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