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Virulence genes and intimin types of Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina

Summary. A total of 153 Shiga-toxin-producing *Escherichia coli* (STEC) isolates from feces of cattle and beef products (hamburgers and ground beef) in Argentina were characterized in this study. PCR showed that 22 (14%) isolates carried *stx*₁ genes, 113 (74%) possessed *stx*₂ genes and 18 (12%) both *stx*₁ and *stx*₂. Intimin (*eae*), enterohemolysin (*ehxA*), and STEC autoagglutinating adhesin (*saa*) virulence genes were detected in 36 (24%), 70 (46%) and in 34 (22%) of the isolates, respectively. None of 34 *saa*-positive isolates carried the gene *eae*, and 31 were *ehxA*-positive. Fourteen (7 of serotype O26:H11 and 4 of serotype O5:H-) isolates had intimin β1, 16 isolates possessed intimin γ1 (11 of serotype O145:H- and 5 of serotype O157:H7), 5 isolates had intimin type ε1 (4 of serotypes O103:H- and O103:H2), and one isolate O111:H- showed intimin type θ/γ2. Although the 153 STEC isolates belonged to 63 different seropathotypes, only 12 accounted for 58% of isolates. Seropathotype ONT:H- *stx*₂ (18 isolates) was the most common, followed by O171:H2 *stx*₂ (12 isolates), etc. The majority (84%) of STEC isolates belonged to serotypes previously found in human STEC and 56% to serotypes associated with STEC isolated from patients with hemolytic uremic syndrome (HUS). Thus, this study confirms that cattle are a major reservoir of STEC pathogenic for humans. To our knowledge, this is the first study that described the presence of *saa* gene in STEC of serotypes O20:H19, O39:H49, O74:H28, O79:H19, O116:H21, O120:H19, O141:H7, O141:H8, O174:H21, and ONT:H21. The serotypes O120:H19 and O185:H7 were not previously reported in bovine STEC. [*Int Microbiol* 2004; 7(4):269-276]

Key words: *Escherichia coli* O157:H7 · intimin · serotypes of STEC · Shiga-toxin-producing *E. coli* · virulence genes

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC), also called verotoxin-producing *E. coli* (VTEC), is the most important recently emerged group of food-borne pathogens. These bacteria can cause severe disease in humans, such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) [3,5,6,17,30]. Cattle, espe-

cially young animals, have been implicated as a principal reservoir of STEC, undercooked ground beef and raw milk being the major vehicles of food-borne outbreaks [2,4,5,7,9,18,20,25-27,34,41]. Argentina has one of the highest recorded frequencies of HUS in the world (300-400 cases/year). It also registers the highest consumption of bovine meat (60 kg/person year) [19].

Human and bovine STEC elaborate two potent phage-encoded cytotoxins, called Shiga-toxins (Stx1 and Stx2) or

verotoxins (VT1 and VT2) [16,30]. In addition to toxin production, another virulence-associated factor expressed by STEC is a protein called intimin, which is responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching and effacing (A/E) lesions in the intestinal mucosa [16]. Intimin is encoded by the chromosomal gene *eae*, which is part of a pathogenicity island termed the locus for enterocyte effacement (LEE) [16]. Severe diarrhea (especially in HC) and HUS are closely associated with STEC types carrying *eae* [17,30]. Differentiation of intimin alleles represents an important tool for STEC typing in routine diagnostics as well as in epidemiological and clonal studies. The C-terminal end of intimin is responsible for receptor binding, and it has been suggested that different intimins may be responsible for different host-tissue cell tropism [42]. Intimin type-specific PCR assays identified 17 variants of *eae* encoding 17 different intimin types and subtypes ($\alpha 1$, $\alpha 2$, $\beta 1$, $\xi R/\beta 2B$, $\delta/\kappa/\beta 2O$, $\gamma 1$, $\theta/\gamma 2$, $\epsilon 1$, $\nu R/\epsilon 2$, ζ , η , $\iota 1$, $\mu R/\iota 2$, λ , μB , νB , ξB) [1,6,8,12,24,36,38,42]. Apart from the capability to produce Shiga toxins and intimins, STEC may have accessory putative virulence factors, such as the enterohemolysin (Ehly), also called enterohemorrhagic *E. coli* hemolysin (EHEC-*HlyA*), which is encoded by *ehxA* [35], and the STEC autoagglutinating adhesin (Saa), encoded by *saa* [14,29].

STEC strains that cause human infections belong to a large number of O:H serotypes (a total of 472 serotypes are listed in the authors' website, <http://www.lugo.usc.es/ecoli>). Most outbreaks of HC and HUS have been attributed to strains of the enterohemorrhagic serotype O157:H7 [4,17,23]. However, as STEC non-O157 are more prevalent in animals and as contaminant in foods, humans are probably more exposed to these strains. Infections with some non-O157 STEC types, such as O26:H11 or H-, O91:H21 or H-, O103:H2, O111:H-, O113:H21, O117:H7, O118:H16, O121:H19, O128:H2 or H-, O145:H28 or H- and O146:H21, are frequently associated with severe illness in humans, but the role of other non-O157 STEC types in human disease needs further examination [2-4,6,10,17,37]. Although more than 400 different O:H serotypes of STEC have been isolated from cattle (a total of 435 serotypes are listed in the authors' website, <http://www.lugo.usc.es/ecoli>), there is a lack of information regarding associations between serotype, intimin types, and virulence factor profiles among bovine STEC isolates [9,11,21,33,40].

Thus, the aim of this study was to establish the serotypes, virulence genes and intimin types of STEC isolated from cattle and beef products in Argentina in order to establish whether bovine STEC have the same serotypes and virulence-factor profiles as STEC strains that cause human infections.

Data from this study have been partly presented as a poster communication at the 5th Int. Symp. on "Shiga toxin (verocytotoxin)-producing *Escherichia coli* infections", Edinburgh, UK [Blanco JE, et al. (2003) Serotypes and virulence genes (*vt1*, *vt2*, *eae*, *ehxA*, *saa*) of VTEC isolated from cattle, food and humans in Argentina. Abstr. P196, p 177].

Materials and methods

***E. coli* isolates and control strains.** A total of 153 STEC isolated as indicated previously [26,27,34] from feces of diarrheic and healthy cattle (n = 131) and beef products (hamburgers and ground beef) (n = 22) in Argentina were characterized in this study. Only one isolate for each animal and food sample was included. *E. coli* strains used as controls were: EPEC-2348 (human, O127:H6, *eae*- $\alpha 1$), AEEC-IH2498a (human, O125:H6, *eae*- $\alpha 2$), EPEC-337 (human, O111:H2, *eae*- $\beta 1$), EPEC-359 (human, O119:H6, *eae*- $\xi R/\beta 2B$), AEEC-6044/95 (human, O118:H5, *eae*- $\delta/\kappa/\beta 2O$), STEC-EDL933 (human, O157:H7, *stx*₁, *stx*₂, *eae*- $\gamma 1$, *ehxA*), STEC-TW07926 (human, O111:H8, *stx*₁, *stx*₂, *eae*- $\theta/\gamma 2$), STEC-VTB-286 (bovine, O103:H2, *stx*₁, *eae*- $\epsilon 1$), AEEC-IH3205a (human, O123:H19, *eae*- $\nu R/\epsilon 2$), STEC-VTO-50 (ovine, O156:H-, *stx*₁, *eae*- ζ), AEEC-CF11201 (human, O125:H-, *eae*- η), AEEC-7476/96 (human, O145:H4, *eae*- $\iota 1$), AEEC-217-2 (human, O101:H-, *eae*- $\mu R/\iota 2$), AEEC-68-4 (human, O34:H-, *eae*- λ), EPEC-373 (human, O55:H51, *eae*- μB), AEEC-IH1229a (human, O10:H-, *eae*- νB), STEC-B49 (bovine, O80:H-, *stx*₁, *eae*- ξB) and K12-185 (negative for *stx*₁, *stx*₂, *eae*, *ehxA*, and *saa*). Strains were stored at room temperature in nutrient broth with 0.75% agar.

Detection of virulence genes by PCR and serotyping. The methodology used for the detection of virulence genes and for serotyping has been described elsewhere [8,9,13]. Base sequences and predicted sizes of amplified products for specific oligonucleotide primers used in this study are shown in Table 1.

Results

Virulence genes. A total of 153 STEC isolates from cattle and beef products in Argentina were characterized in this study. PCR showed that 22 (14%) isolates carried *stx*₁ genes, 113 (74%) possessed *stx*₂ genes and 18 (12%) both *stx*₁ and *stx*₂. Intimin (*eae*), enterohemolysin (*ehxA*), and STEC autoagglutinating adhesin (*saa*) virulence genes were detected in 36

Table 1. PCR primer and conditions for amplification of virulence genes

Gene	Primer	Oligonucleotide sequence (5'-3')	Fragment size (bp)	Annealing temperature (°C)	Primer coordinates	GenBank accession number	Reference
<i>stx</i> ₁	VT1-A	CGCTGAATGTCATTCGCTCTGC	302	55°C	113-134	M17358	Blanco et al. [9]
	VT1-B	CGTGGTATAGCTACTGTCCACC			394-414		
<i>stx</i> ₂	VT2-A	CTTCGGTATCCTATTCCTCCGG	516	55°C	50-69	M59432	Blanco et al. [9]
	VT2-B	CTGCTGTGACAGTGACAAAAACGC			543-565		
<i>ehxA</i>	HlyA1	GGTGCAGCAGAAAAAGTTGTAG	1551	60°C	238-259	X79839	Schmidt et al. [35]
	HlyA4	TCTCGCCTGATGATTTTGGTA			1767-1788		
<i>saa</i>	SAA-DF	CGTGATGAACAGGCTATTGC	119	66°C	1423-1442	AF399919	Paton & Paton [29]
	SAA-DR	ATGGACATGCCTGTGGCAAC			1522-1541		
<i>eae</i> ^a	EAE-1	GGAACGGCAGAGGTTAATCTGCAG	775	55°C	1441-1460	AF022236	Blanco et al. [9]
	EAE-2	GGCGCTCATCATAGTCTTTC			2193-2215		
<i>eae-α1</i>	EAE-FB	AAAACCGCGGAGATGACTTC	820	60°C	1909-1928	AF022236	Blanco et al. [9]
	EAE-A	CACTCTTCGCATCTTGAGCT			2709-2728		
<i>eae-α2</i>	IH2498aF	AGACCTTAGGTACATTAAGTAAGC	517	60°C	2099-2122	AF530555	Blanco et al. [9]
	IH2498aR	TCCTGAGAAGAGGGTAATC			2597-2615		
<i>eae-β1</i>	B1A	ACTTCGCCACTTAATGCCAGC	730	66°C	1924-1944	AF453441	This study
	B1B	TTGCAGCACCCCATGTTGAAT			2633-2653		
<i>eae-ξR/β2B</i> ^{b,c}	B2A	AAGGGGGAAACCCCTGTGTA	604	66°C	2056-2076	AF530556	This study
	B2B	ATTTATTCGCAGCCCCCACG			2639-2659		
<i>eae-δ/κ/β2O</i> ^c	EAE-FB	AAAACCGCGGAGATGACTTC	833	60°C	1909-1928	U66102	Blanco et al. [9]
	EAE-D	CTGTATACACCCGATGGTAAC			2721-2741		
<i>eae-γ1</i>	EAE-FB	AAAACCGCGGAGATGACTTC	804	60°C	1909-1928	AF071034	Blanco et al. [9]
	EAE-C1	AGAACGCTGCTCACTAGATGTC			2691-2712		
<i>eae-θ/γ2</i> ^d	EAE-FB	AAAACCGCGGAGATGACTTC	808	58°C	1909-1928	AF025311	Blanco et al. [9]
	EAE-C2	CTGATATTTATCAGCTTCA			2697-2716		
<i>eae-ε1</i>	EAE-FB	AAAACCGCGGAGATGACTTC	722	66°C	1909-1928	AF116899	Blanco et al. [9]
	LP5	AGCTCACTCGTAGATGACGGCAAGCG			2605-2630		
<i>eae-νR/ε2</i> ^e	EAE-E2F	AATACAGAAGTTAAGGCAT	378	58°C	2230-2248	AF530554	This study
	EAE-E2R	ACGACCACTAATTCATTTTC			2590-2607		
<i>eae-ζ</i>	Z1	GGTAAGCCGTTATCTGCC	206	62°C	2062-2079	AF449417	Blanco et al. [9]
	Z2	ATAGCAAGTGGGGTGAAG			2250-2267		
<i>eae-η</i>	EAE-FB	AAAACCGCGGAGATGACTTC	702	60°C	1899-1918	AJ308550	This study
	ETA-B	TAAGCGACCACTATTCGTG			2582-2600		
<i>eae-ι1</i>	EAE-FB	AAAACCGCGGAGATGACTTC	651	55°C	1909-1928	AJ308551	This study
	IOTA-B	GTCATATTTAACTTTTACACTA			2538-2559		
<i>eae-μR/ι2</i> ^e	Iota2-F	CTGGTAAAGCGATATGTCAAAC	936	58°C	1850-1870	AF530553	This study
	Iota2-R	GCGTTTTTGAAGAAACATTTTGC			2763-2785		
<i>eae-λ</i>	68.4F	CGGTCAGCCTGTGAAGGGC	466	64°C	2061-2079	AJ715409	Blanco et al. [9]
	68.4R	ATAGATGCCTCTTCCGGTATT			2506-2526		
<i>eae-μB</i> ^f	EAE-FB	AAAACCGCGGAGATGACTTC	665	60°C	1909-1928	AJ705049	This study
	FV373R	ACTCATCATAATAAGCTTTTGG			2541-2563		
<i>eae-νB</i> ^f	IH1229aF	CACAGCTTACAATTGATAACA	311	60°C	269-289	AJ705050	Blanco et al. [9]
	IH1229aR	CTCACTATAAGTCATACGACT			559-579		
<i>eae-ξB</i> ^f	EAE-FB	AAAACCGCGGAGATGACTTC	468	66°C	1909-1928	AF116899	Blanco et al. [9]
	B49R	ACCACCTTTAGCAGTCAATTTG			363-384		

^aUniversal oligonucleotide primer pair EAE1 and EAE-2 with homology to the 5' conserved region of *eae* (detects all types of *eae* variants described thus far). Primers used to detect *eae*.

^bThe intimin β2 of Blanco et al. [9] is identical to intimin ξ described by Ramachandran et al. [32]. In this study, our β2 is referred as ξR/β2B.

^cThe intimin δ of Adu-Bobie et al. [1] is identical to intimin K of Zhan et al. [42]. The intimin δ of Adu-Bobie et al. [1] was also termed β2 by Oswald et al. [24]. Thus, in the present study this intimin is referred to as δ/κ/β2O.

^dThe intimin θ of Tarr and Whittam [38] is the same as intimin γ2 of Oswald et al. [24].

^eIntimins μ, ν, and ξ described by Ramachandran et al. [32] in ruminant *E. coli* strains. When Ramachandran et al. [32] submitted the nucleotide sequences of the μ, ν, and ξ genes, these intimin types were designated ι2, ε2 and β2, respectively.

^fIntimins μ, ν, and ζ described by Blanco et al. [9, unpublished results].

(24%), 70 (46%) and in 34 (22%) of the isolates, respectively. None of 34 *saa*-positive isolates carried *eae*, and 31 were *ehxA* positive (Table 2).

Serotypes and seropathotypes. STEC isolates belonging to 33 O serogroups and 47 O:H serotypes were identified.

However, 57% were of one of the following 12 serogroups (O5, O20, O26, O39, O91, O103, O113, O117, O145, O157, O171, and O174) and 69% of the isolates belonged to only 13 serotypes (O5:H-, O20:H19, O26:H11, O39:H49, O91:H21, O113:H21, O117:H7, O145:H-, O157:H7, O171:H2,

Table 2. Seropathotypes (serotypes and virulence genes) of Shiga-toxin-producing *Escherichia coli* (STEC) isolates (n = 153)

Serotype	No. (origin) ^d	<i>stx</i> ₁	<i>stx</i> ₂	<i>eae</i>	<i>ehxA</i>	<i>saa</i>	Serotype	No. (origin) ^d	<i>stx</i> ₁	<i>stx</i> ₂	<i>eae</i>	<i>ehxA</i>	<i>saa</i>
O2:H5 ^b	1 (g)	-	+	-	-	-	O117:H7 ^a	7 (2g, 3f, 2m)	-	+	-	-	-
O2:H25	1 (f)	-	+	-	-	-	O118:H16 ^b	1 (g)	+	-	β1	+	-
O5:H- ^b	4 (g)	+	-	β1	+	-	O120:H19 ^c	1 (f)	-	+	-	+	+
O5:H27	1 (g)	+	-	-	+	-	O141:H7	1 (g)	+	+	-	+	+
O8:H16	2 (f, m)	+	-	-	-	+	O141:H8 ^a	1 (g)	+	+	-	+	+
O8:H19 ^b	1 (m)	-	+	-	+	-	O141:H8 ^a	1 (g)	-	+	-	+	+
O15:H21	1 (f)	-	+	-	-	-	O145:H- ^b	2 (f)	+	-	γ1	+	-
O20:H7 ^a	2 (g)	+	+	-	-	-	O145:H- ^b	9 (f)	-	+	γ1	+	-
O20:H19 ^b	4 (2g, 1f, 1m)	+	+	-	+	+	O157:H7 ^b	5 (f)	-	+	γ1	+	-
O20:H19 ^b	1 (g)	+	+	-	-	-	O162:H7	1 (m)	-	+	-	-	-
O20:H19 ^b	1 (g)	-	+	-	+	+	O165:H- ^b	1 (g)	-	+	ε1	+	-
O20:H19 ^b	1 (m)	-	+	-	-	-	O168:H8	1 (g)	-	+	-	-	-
O20:H?	1 (g)	+	-	-	-	-	O171:H- ^b	3 (f)	-	+	-	-	-
O22:H8 ^b	2 (m)	+	+	-	+	+	O171:H2 ^a	12 (4g, 7f, 1m)	-	+	-	-	-
O25:H19	1 (f)	-	+	-	-	-	O174:H21 ^b	1 (g)	+	+	-	+	+
O26:H11 ^b	5 (g)	+	-	β1	+	-	O174:H21 ^b	1 (f)	+	-	-	-	-
O26:H11 ^b	2 (g)	-	+	β1	+	-	O174:H21 ^b	8 (4g, 3f, 1m)	-	+	-	-	-
O38:H39	1 (g)	+	-	β1	+	-	O175:H8	2 (f)	-	+	-	-	-
O39:H49	1 (g)	+	+	-	+	+	O177:H- ^a	1 (g)	-	+	β1	+	-
O39:H49	4 (g)	-	+	-	+	+	O178:H19	2 (f)	-	+	-	-	-
O74:H28	1 (g)	-	+	-	+	+	O185:H7 ^c	1 (f)	-	+	-	-	-
O79:H19	1 (g)	-	+	-	+	+	ONT:H- ^b	18 (8g, 9f, 1m)	-	+	-	-	-
O88:H21	1 (m)	+	+	-	+	-	ONT:H2 ^a	1 (m)	-	+	-	-	-
O91:H21 ^b	4 (1g, 2f, 1m)	-	+	-	+	+	ONT:H8 ^a	1 (m)	+	+	-	+	+
O103:H- ^b	1 (g)	+	+	ε1	+	-	ONT:H8 ^a	1 (g)	+	-	-	-	-
O103:H- ^b	1 (g)	-	+	ε1	+	-	ONT:H16 ^b	1 (m)	+	-	-	-	+
O103:H2 ^b	1 (g)	+	+	ε1	+	-	ONT:H21 ^a	1 (g)	-	+	-	+	-
O103:H2 ^b	1 (f)	+	-	ε1	+	-	ONT:H21 ^a	9 (4g, 4f, 1m)	-	+	-	-	-
O111:H- ^b	1 (g)	+	-	θ/γ2	+	-	ONT:H21 ^a	1 (g)	-	+	-	+	+
O113:H21 ^b	3 (2f, 1m)	-	+	-	-	-	ONT:H21 ^a	1 (f)	+	+	-	+	+
O113:H21 ^b	2 (1g, 1m)	-	+	-	+	+	ONT:HNT	1 (m)	-	+	-	-	+
O116:H21 ^a	2 (1g, 1m)	-	+	-	+	+							

^a Serotypes previously found in human STEC.

^b Serotypes previously associated with human STEC that cause hemolytic uremic syndrome (HUS).

^c New serotype not found in bovine STEC in previous studies.

^d Origin; g, grazing cattle; f, cattle in feedlot; m, meat.

O174:H21, ONT:H- and ONT:H21). The serotypes O120:H19 and O185:H7 were not previously reported in bovine STEC.

Although the 153 STEC isolates belonged to 63 different seropathotypes (associations between serotypes and virulence genes), only 12 accounted for 58% of isolates. Seropathotype ONT:H- *stx*₂ (18 isolates) was the most common, followed by O171:H2 *stx*₂ (12 isolates), ONT:H21 *stx*₂ (9 isolates), O145:H- *stx*₂ *eae*-γ1 *ehxA* (9 isolates), O174:H21 *stx*₂ (8 isolates), O117:H7 *stx*₂ (7 isolates), O26:H11 *stx*₁ *eae*-β1 *ehxA* (5 isolates) and O157:H7 *stx*₂ *eae*-γ1 *ehxA* (5 isolates).

Gene *saa* was detected in *eae*-negative strains of serotypes O8:H16, O20:H19 (5 isolates), O22:H8, O39:H49 (5 isolates), O74:H28, O79:H19, O91:H21 (4 isolates), O113:H21, O116:H21, O120:H19, O141:H7, O141:H8, O174:H21, ONT:H8, ONT:H16, ONT:H21 and ONT:HNT.

Typing of *eae* (intimin) genes. Fourteen isolates (7 of serotype O26:H11 and 4 of serotype O5:H-) had intimin β1, 16 isolates possessed intimin γ1 (11 of serotype O145:H- and 5 of serotype O157:H7), 5 isolates had intimin type ε1 (4 of serotypes O103:H- and O103:H2), and one isolate, O111:H-, showed intimin type θ/γ2 (Table 2).

Discussion

In view of the increasing importance of STEC as emerging food-borne pathogens and reports on O157:H7 and non-O157 STEC causing severe illness, evaluation of associated virulence factors of such isolates is required. In the present work, we established the serotypes, virulence genes and

intimin types of STEC isolated from cattle and beef products in Argentina in order to determine whether bovine STEC possess the same serotypes and virulence-factor profiles as STEC isolates that cause human infection. To our knowledge, this study is the first to document the detection of *saa* and to have carried out intimin typing in STEC isolated in Argentina.

It should be noted that the majority (84%) of the 153 bovine STEC isolates characterized in this study belonged to serotypes previously found in human STEC, and 56% to serotypes associated with STEC isolated from patients with HUS. Thus, this study confirms that cattle are a major reservoir of STEC pathogenic for humans. Similarly, Meichtri et al. [20] found in Argentina 86 bovine STEC isolates belonging to 34 serotypes, 17 of which had been previously associated with human disease. The typeable bovine STEC isolated by Meichtri et al. [20] belonged to 17 O serogroups, and, in order of frequency, were: O8 (16 isolates), O113 (14), O103 (5), O91 (4), O171 (3), O174 (3), O25 (2), O112 (2), O145 (2), O2 (1), O11 (1), O104 (1), O121 (1), O128 (1), O143 (1), O146 (1) and O157 (1). The serotype O8:H19 was the most prevalent (13%) [20].

In Spain, Blanco et al. [9] found that 514 STEC isolates belonged to 66 O serogroups and 113 O:H serotypes. However, 67% were of one of these 15 serogroups (O2, O4, O8, O20, O22, O26, O77, O91, O105, O113, O116, O157, O171, O174, and O177) and 52% of the isolates belonged to only 10 serotypes (O4:H4, O20:H19, O22:H8, O26:H11, O77:H41, O105:H18, O113:H21, O157:H7, O171:H2, and O113:H19), including 10 serotypes also found among STEC that cause human infections and six serotypes associated with HUS (O20:H19, O22:H8, O26:H11, O105:H18, O113:H21, and O157:H7). Likewise, among the 20 serotypes most frequently isolated in cattle or cattle products in Canada by Johnson et al. [15], 18 were isolated from humans, and 11 of those were serotypes associated with bloody diarrhea/hemorrhagic colitis and/or HUS. Pradel et al. [31] in France, Beutin et al. [2], and Montenegro et al. [22] in Germany, and Wells et al. [39] in the USA also found that many STEC recovered from cattle belonged to serotypes previously associated with human disease. Along with other authors [11,15,21,40], we observed that bovine and human STEC of the same serotype have similar known virulence-associated properties. Of the 153 STEC isolates serotyped in the present study, 35 (23%) were considered O non-typeable (ONT) with all available O antisera (O1 to O185). This highlights the need to extend the serotyping scheme to include new and emerging STEC serogroups. Our study also identified two new serotypes (O120:H19 and O185:H7) not previously reported in bovine STEC strains.

Gene *eae*, which has been shown to be necessary for attaching and effacing activity, encodes a 94- to 97-kDa outer-membrane protein (OMP), which is termed intimin [16]. Many investigators have underlined the strong association between carrying *eae* and the capacity of STEC to cause severe human disease, especially HUS [1,3,6,24,30]. This important virulence gene was detected in 100% of STEC O157:H7 and in 21% of non-O157 bovine isolates assayed in the present study. A similar prevalence of the intimin gene has also been found in other studies [4,9]. Nevertheless, the production of intimin is not essential for pathogenesis, because a number of sporadic cases of HUS have been caused by *eae*-negative non-O157 STEC isolates. Thus, STEC O104:H21 and O113:H21 isolates lacking *eae* were responsible for an outbreak and a cluster of three HUS cases in the USA and Australia, respectively [28,30]. Paton and Paton [29] recently described a novel autoagglutinating adhesin, designated Saa, in an *eae*-negative O113:H21 STEC isolate responsible for an outbreak of HUS. In our study, the *saa*-positive STEC isolates (22% of all isolates) were all *eae*-negative and often *ehxA*-positive. However, a recent study showed that there is no significant association between STEC isolated from patients with HUS and *saa* [14]. Nevertheless, the possible role of *saa* in human infection remains controversial. Further studies are required to elucidate the contribution of this gene to milder human gastrointestinal conditions, such as diarrhea. However, in the present and in previous studies [14,43], *saa* was frequently found in bovine and ovine STEC, suggesting that Saa may have a role in the attachment to bovine and ovine gut. To our knowledge, this is the first study to describe the association of *saa* with serotypes O20:H19, O39:H49, O74:H28, O79:H19, O116:H21, O120:H19, O141:H7, O141:H8, O174:H21, and O113:H21.

Analysis of the nucleotide sequences of the intimin genes from different STEC and enteropathogenic *E. coli* (EPEC) strains has shown a high degree of homology in the 5' two-thirds of the genes and a significant degree of heterogeneity in the 3' one-third of the genes [1,12]. Seventeen variants of *eae* were identified by intimin type-specific PCR assays using oligonucleotide primers complementary to the 3'-end of the specific intimin genes that encode for the intimin types and subtypes α 1, α 2, β 1, ξ R/ β 2B, δ / κ / β 2O, γ 1, θ / γ 2, ϵ 1, ν R/ ϵ 2, ζ , η , ι 1, μ R/ ι 2, λ , μ B, ν B, ξ B [1,6,8,24,32,36,38,42]. As in human strains, the intimin types β 1 and γ 1 are the most frequently found among bovine STEC isolates. Intimin β 1 was mainly found among strains belonging to serotypes O5:H- and O26:H11, whereas intimin type γ 1 was detected in all STEC isolates of serotypes O145:H- and O157:H7 assayed.

The highly virulent seropathotypes O26:H11 *stx*₁ or *stx*₂ *eae*-β1 *ehxA* (7 isolates), O103:H2/ H- *stx*₁ and/or *stx*₂ *eae*-ε1 *ehxA* (4 strains), O118:H16 *stx*₁ *eae*-β1 *ehxA* (1 isolate), O145:H- *stx*₁ or *stx*₂ *eae*-γ1 *ehxA* (11 isolates), and O157:H7 *stx*₂ *eae*-γ1 *ehxA* (5 isolates) were detected in 28 bovine STEC characterized in the present study. Although our results and those of other authors indicate that STEC strains of human and animal origin with the same serotype are similar with respect to the presence of known virulence-associated factors [2,10,11,21,33], the results of Boerlin et al. [10] suggest that STEC isolates from humans form a different population from those found in the bovine reservoir or that they are only a subpopulation of the latter. Thus, future studies are necessary to establish whether animal and human strains represent the same clones or are only related subpopulations.

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Genes de virulencia y tipos de intiminas de *Escherichia coli* productoras de toxinas Shiga aisladas de ganado bovino y carne de vacuno en Argentina

Resumen. En este estudio hemos caracterizado un total de 153 *Escherichia coli* productores de toxinas Shiga (STEC) aisladas de las heces de ganado bovino y de carne picada y hamburguesas de vacuno en Argentina. Los ensayos de PCR mostraron que 22 (14%) aislamientos llevaban el gen *stx₁*, 113 (74%) presentaban el gen *stx₂* y que 18 (12%) tenían ambos genes. Los genes de virulencia para la intimina (*eae*), la enterohemolisina (*ehxA*) y la adhesina autoaglutinante de STEC (*saa*) fueron detectados en 36 (24%), 70 (46%) y 34 (22%) de los aislamientos, respectivamente. Ninguno de los 34 aislamientos *saa*-positivos llevaba el gen *eae*, pero 31 eran *ehxA*-positivos. Catorce aislamientos (7 del serotipo O26:H11 y 4 del serotipo O5:H-) tenían la intimina β 1, 16 poseían la intimina γ 1 (11 del serotipo O145:H- y 5 del serotipo O157:H7), 5 aislamientos eran positivos para la intimina tipo ϵ 1 (4 de los serotipos O103:H- y O103:H2), y un aislamiento O111:H- mostró la intimina tipo θ/γ 2. Aunque los 153 aislamientos de STEC pertenecían a 63 seropatótipos, sólo 12 constituían el 58% de los ais-

Genes de virulencia e tipos de intiminas de *Escherichia coli* produtoras de toxinas Shiga aislados de gado bovino e carne bovina na Argentina

Resumo. Neste estudo, um total de 153 *Escherichia coli* produtoras de toxinas Shiga (STEC) isoladas de fezes de gado bovino e de carne moída e hamburguesas de carne bovina na Argentina foram caracterizados. Os ensaios de PCR mostraram que 22 (14%) dos isolados possuíam o gene *stx₁*, 113 (74%) o gene *stx₂*, e que 18 (12%) tinham ambos os genes. Os genes de virulência para a intimina (*eae*), a enterohemolisina (*ehxA*) e a adesina autoaglutinante de STEC (*saa*) foram detectados em 36 (24%), 70 (46%) e 34 (22%) dos isolados, respectivamente. Nenhum dos 34 isolados *saa*-positivos tinham o gene *eae*, no entanto 31 eram *ehxA*-positivos. Catorze isolados (7 do serótipo O 26:H11 e 4 do serótipo O5:H-) tinham a intimina β 1, 16 possuíam a intimina γ 1 (11 do serótipo O145:H- e 5 do serótipo O157:H7), 5 isolados foram positivos para a intimina tipo ϵ 1 (4 dos serótipos O103:H- e O103:H2), e um isolado O111:H- apresentou a intimina tipo θ/γ 2. Enquanto os 153 isolados de STEC pertenciam a 63 seropatótipos, somente 12 representaram 58% dos isolados. O seropatótipo O111:H- *stx₂*

lamientos. El serotipo ONT:H- *stx*₂ (18 aislamientos) fue el más común, seguido por el O171:H2 *stx*₂ (12 aislamientos), etc. La mayoría de los aislamientos (84%) de STEC pertenecían a serotipos encontrados previamente en seres humanos y el 56% a serotipos asociados con STEC aislados de pacientes con el síndrome urémico hemolítico (HUS). Por tanto, este estudio confirma que el ganado bovino es un importante reservorio de STEC patógenos para humanos. Según nuestra información, este es el primer estudio que describe la presencia del gen *saa* en STEC de los serotipos O20:H19, O39:H49, O74:H28, O79:H19, O116:H21, O120:H19, O141:H7, O141:H8, O174:H21, y ONT:H21. Los serotipos O120:H19 y O185:H7 tampoco habían sido descritos previamente en STEC de origen bovino. [Int Microbiol 2004; 7(4):269-276]

Palabras clave: *Escherichia coli* O157:H7 · intimina · serotipos de STEC · *E. coli* productoras de toxina Shiga · genes de virulencia

(18 isolados) foi o mais comum, seguido pelo O171:H2 *stx*₂ (12), etc. A maioria dos isolados (84%) de STEC pertenciam à serótipos encontrados previamente em seres humanos e cerca de 56% à serótipos associados com STEC isolados de pacientes com a síndrome urémica hemolítica (HUS). Esses dados confirmam que o gado bovino é um importante reservatório de STEC patogênicos para humanos. Segundo nossa informação, este é o primeiro estudo que descreve a presença do gene *saa* em STEC dos serótipos O20:H19, O39:H49, O74:H28, O79:H19, O116:H21, O120:H19, O141:H7, O141:H8, O174:H21, e ONT:H21. Os serótipos O120:H19 e O185:H7 também não haviam sido descritos previamente em STEC de origem bovina. [Int Microbiol 2004; 7(4):269-276]

Palavras chave: *Escherichia coli* O157:H7 · intimina · serótipos de STEC · *E. coli* produtoras de toxina Shiga · genes de virulência