

INTRODUCTION: Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic food- and waterborne pathogen that cause uncomplicated diarrhea, bloody diarrhea (BD), and hemolytic uremic syndrome (HUS). The O157:H7 is the most common STEC serotype and is usually involved in large outbreaks. In Argentina, the STEC-associated diseases are a serious public health concern because the number of affected people. Surveillance using molecular epidemiology techniques such as pulsed-field gel electrophoresis (PFGE) is used to establish relatedness of STEC isolates and has frequently been applied in epidemiologic investigations of outbreaks or sporadic cases in order to establish relatedness between isolates of different origins. National and International *E.coli* O157 databases have been created. Since 2005, the Public Health Region VIII has consolidated the information of *E.coli* O157 through the strategy of sentinel surveillance unit of the National System of Health Surveillance (SNVS).

OBJETIVE: The aim of this study was to establish the genetic diversity and to estimate the clonal relatedness of *E.coli* O157:H7 strains isolated from HUS and BD cases and asymptomatic household contacts (AC) living in different cities along the coast of the province of Buenos Aires between 1996 and 2007.

MATERIALS AND METHODS:

Bacterial strains: Thirty-four *E.coli* O157:H7 strains isolated from HUS (21), BD (7), and asymptomatic household contacts (6) were included.

Biotyping, serotyping and phage typing: Biochemical identification and serotyping were performed as described previously (Physiopathogeny Service, Department Bacteriology, INEI-ANLIS "Carlos G. Malbrán" (2003). The phage typing was performed by the method described by Amhed et al. (1987) and extended by Khakhria et al. (1990).

Genotypic characterization: The *stx1*, *stx2*, *rfbO157*, *eae*, *ehxA* and *fliCh7* genes were characterized by PCR (Pollard et al.(1990), Gannon et al.(1993) and Schmidt et al.(1995). Genotyping of *stx1* and *stx2* was performed by RFLP-PCR as described by Zhang et al. (2002) and Tyler et al. (1991), respectively.

XbaI-PFGE: Macrorestriction fragment analysis by PFGE was performed using the 24-h PulseNet standardized PFGE protocol for *E.coli* O157:H7 with minor modifications. The clonal relatedness among isolates was established using the BioNumerics software version 4.0 (Applied Maths, Kortrijk, Belgium).

RESULTS: The frequency of *stx* genotypes and the prevalent phage types (PT) are shown in Figures 1 and 2. All strains were *eae* and *ehxA*-positive.

By *XbaI*-PFGE, a total of 26 different patterns were identified among the 34 *E.coli* O157 strains within at least 65.7% of similarity. Five clusters of isolates with identical *XbaI* profiles were found: #1, AREXHX01.0011 (4 strains), #2 AREXHX01.0022 (3 strains), #3, AREXHX01.0139 (2 strains), #4, AREXHX01.0200 (2 strains) and #5, AREXHX01.0449 (2 strains) (Figure 3). Some isolates were also indistinguishable when tested with *BlnI* as second enzyme.

Figure 1:
Frecuency of *stx* genotypes of *E.coli* O157:H7 (N=34)

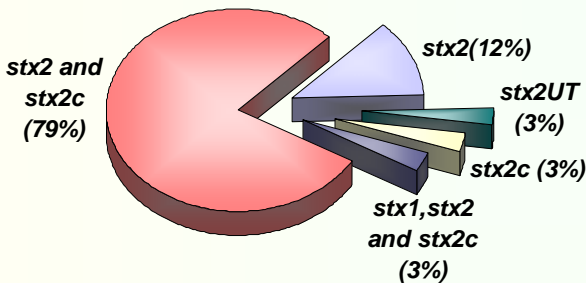


Figure 2:
Prevalent phage types of *E.coli* O157:H7 (N=34)

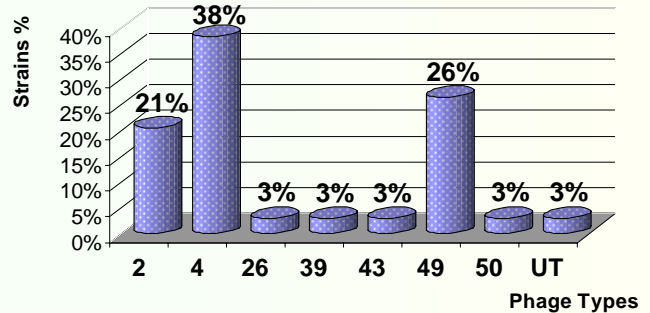


Figure 3:
Clonal Relatedness of *E.coli* O157:H7 strains (N=34)

Dice (Opt=1.50%) (Tot 1.5%-1.5%) (H=0.0% S=0.0%) [0.0%-98.3%]

PFGE-XbaI	Nº FP	XbaI-PFGE	Serotipo	Genotipo	Fagotipo	Origen	Procedencia	Año
	678/04	AREXHX01.0094	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	2004
	772/03	AREXHX01.0153	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	2003
	648/05	AREXHX01.0022	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	2005
	140/97	AREXHX01.0022	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Tuyú	1997
	35/96	AREXHX01.0022	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	1996
	498/05	AREXHX01.0014	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	DS	Mar del Plata	2005
	24/97	AREXHX01.0041	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	UT	SUH	Sar Bernardo	1997
	580/04	AREXHX01.0370	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	DS	Mar del Plata	2004
	773/03	AREXHX01.0183	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	2003
	400/03	AREXHX01.0255	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	49	SUH	Mar del Plata	2003
	412/01	AREXHX01.0200	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Chamendi	2001
	643/06	AREXHX01.0200	E. coli O157:H7	eae+/Ehlye/stx2 only	4	DS	Mar del Plata	2006
	177/06	AREXHX01.0139	E. coli O157:H7	eae+/Ehlye/stx1/stx2 y stx2c(stx2vh-a)	4	SUH	Mar del Plata	2006
	924/06	AREXHX01.0139	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Madariaga	2006
	403/05	AREXHX01.0365	E. coli O157:H7	eae+/Ehlye/stx2 only	43	DS	Mar del Plata	2005
	402/05	AREXHX01.0011	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Loberia	2005
	404/04	AREXHX01.0011	E. coli O157:H7	eae+/Ehlye/stx2 only	4	SUH	Mar del Plata	2004
	404/06	AREXHX01.0011	E. coli O157:H7	eae+/Ehlye/UT	25	DS	Mar del Plata	2006
	424/06	AREXHX01.0011	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	CA	Mar del Plata	2006
	775/03	AREXHX01.0279	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	DS	Mar del Plata	2003
	302/07	AREXHX01.0023	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Mar del Plata	1996
	306/07	AREXHX01.0448	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Chapadmalal	1996
	225/07	AREXHX01.0038	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Mar del Plata	1997
	1065/01	AREXHX01.0109	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	S/O	2001
	800/05	AREXHX01.0105	E. coli O157:H7	eae+/Ehlye/stx2	2	CA	Mar del Plata	2005
	859/06	AREXHX01.0418	E. coli O157:H7	eae+/Ehlye/stx2c(stx2vh-a)	39	DS	Mar del Plata	2006
	946/01	AREXHX01.0178	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	CA	Balcarce	2001
	308/07	AREXHX01.0442	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	50	CA	Mar del Plata	1997
	303/07	AREXHX01.0445	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	4	SUH	Mar del Plata	1996
	280/07	AREXHX01.0449	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	SUH	Mar del Plata	2007
	392/07	AREXHX01.0449	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	SUH	Mar del Plata	2007
	291/07	AREXHX01.0448	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	SUH	Mar de Ajo	2007
	391/07	AREXHX01.0447	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	CA	Mar del Plata	2007
	1066/01	AREXHX01.0188	E. coli O157:H7	eae+/Ehlye/stx2 y stx2c(stx2vh-a)	2	SUH	Villa Gesell	2001

CONCLUSIONS: PFGE was useful to genetically subtype isolates, which demonstrated great diversity. However, some strains isolated in different cities throughout several years were grouped in clusters and may be clonal groups. However, no obvious epidemiologic linkage could be demonstrated among them.

Using standardized detection and typing tools, reference laboratories will be able to detect the emergence of potentially hypervirulent clones and monitor their spread.

This study also allowed to create a local database to help in the early detection of diffuse outbreaks.

REFERENCES: Rivas M, Miliwebsky E, Chinen I, Roldán CD, Balbi L, García B, Fiorilli G, Sosa-Estani S, Kincaid J, Griffín PM and the Case-Control Study Group. Characterization and epidemiologic subtyping of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. 2006. Estados Unidos. Foodborne Pathogens and Disease 3: 88-96. ISSN: 1535-3141. Inglés.