Intraserotype diversity among Argentinian verocytotoxigenic *Escherichia coli* detected by random amplified polymorphic DNA analysis

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Most cases of diarrhoea-associated haemolytic uraemic syndrome (HUS) are caused by verocytotoxin-producing *Escherichia coli* (VTEC). Argentina has the highest worldwide incidence of HUS, but with a lower incidence of VTEC O157 : H7 serotype than non-Latin American countries. A large number of VTEC serotypes have been isolated from cattle and cattle-derived food products in Argentina. The aim of this work was to study intraserotype genetic diversity among these VTEC strains by random amplification of polymorphic DNA (RAPD). Strains were selected that belonged to the same serotype, but had been isolated from different sources (cattle and meat). Intraserotype genetic diversity was detected among strains belonging to O20 : H19, O113 : H21, O117 : H7, O157 : H7, O171 : H2 and O174 : H21, but only one RAPD profile corresponded to strains belonging to O91 : H21, although these isolates were from different sources.

INTRODUCTION

Verocytotoxin-producing *Escherichia coli* (VTEC) are human pathogens that cause a wide spectrum of illness, encompassing asymptomatic infection, mild-to-moderate diarrhoea, haemorrhagic colitis, and haemolytic uraemic syndrome (HUS) (Karmali, 1989). Argentina has the highest worldwide incidence of HUS (López *et al.*, 1998), corresponding to 10·5 per 100 000 in children under 5 years of age (Meichtri *et al.*, 2004). VTEC serotypes appear to differ in pathogenic potential, but the molecular basis for this is not known (Karmali, 2005). Although serotype O157 : H7 has been implicated in most cases of HUS globally, there is growing concern about non-O157 serotypes of VTEC (Karmali, 2005). In Argentina, the incidence of O157 : H7 is low in HUS patients compared with that of non-Latin American countries (López *et al.*, 1998). Several other serotypes (O26 : H11, O91 : H−, O103 : H-, O111 : H-, O113 : H21, O121 : H19 and O145 : H−) have also been isolated from human patients in Argentina (Guth *et al.*, 2003).

Domestic animals, particularly bovines, appear to be the major reservoir of VTEC, and foods of animal origin are the major sources of human infection (Karmali, 1989). A large number of VTEC serotypes have been isolated from cattle and cattle-derived food products in Argentina (Parma *et al.*, 2000; Chinen *et al.*, 2001; Padola *et al.*, 2002, 2004; Blanco *et al.*, 2004; Meichtri *et al.*, 2004). In this study, our objective was to characterize VTEC isolates by random amplification of polymorphic DNA (RAPD) to analyse the genetic diversity among strains belonging to the same serotype that had been isolated from both cattle and meat. This is not an epidemiological study, as strains isolated from meat are not epidemiologically linked to those isolated from cattle.

Most RAPD studies of VTEC focus on serogroup O157 (Bielaszewska *et al.*, 1998; Hopkins & Hilton, 2001; Tutenel *et al.*, 2003; Kim *et al.*, 2005). Only a few VTEC non-O157 serogroups have been analysed by RAPD: O26, O103 and O111 (Schmidt *et al.*, 1999; Peixoto *et al.*, 2001), and we have previously analysed isolates belonging to O145 (Padola *et al.*, 2002). Therefore, this paper is one of the few that study intraserotype diversity by RAPD among both O157 and non-O157 serogroups.

Abbreviations: RAPD, random amplification of polymorphic DNA; VTEC, verocytotoxin-producing *Escherichia coli*.

METHODS

Bacterial isolates. The VTEC strains used in this study were isolated from cattle and from bovine meat, and most of them have been described previously (Parma *et al.*, 2000; Padola *et al.*, 2004; Blanco *et al.*, 2004). Detection was by PCR analysis of the genes encoding verocytotoxin 1 and 2 (vt_1 and vt_2), intimin (*eae*), enterohaemolysin (*ehxA*) and Shiga toxin-producing *Escherichia coli* (STEC) autoagglutinating adhesin (*saa*). When two or more isolates from the same sample did not differ in either one virulence factor or the serogroup, only one of them was analysed in this study. We selected strains that belonged to the same serotype but had been isolated from different sources (cattle and meat). They belonged to serotypes O20 : H19 (seven isolates), O91 : H21 (four isolates), O113 : H21 (four isolates), O117 : H7 (nine isolates), O157 : H7 (six

Table 1. VTEC isolates analysed in this study

Serotype	Strain name (origin)*	Virulence profile†	RAPD profile
O20 : H19	T 22-1 (g)	vt_1^+ vt_2^+	A
	T 22-2 (g)	vt_1^+ vt_2^+ *ehxA*$^+$ *saa*$^+$	A
	HT 1-6 (h)	vt_1^+ vt_2^+ *ehxA*$^+$ *saa*$^+$	B
	AM 114-1 (a)	vt_1^+ vt_2^+ *ehxA*$^+$ *saa*$^+$	B
	AP 28-1 (g)	vt_2^+ *ehxA*$^+$ *saa*$^+$	B
	FO 114 (f)	vt_1^+ vt_2^+ *ehxA*$^+$ *saa*$^+$	B
	HT 6-2 (h)	vt_2^+	C
O91 : H21	AP 16-1 (g)	vt_2^+ *ehxA*$^+$ *saa*$^+$	D
	FO 130 (f)	vt_2^+ *ehxA*$^+$ *saa*$^+$	D
	FO 135 (f)	vt_2^+ *ehxA*$^+$ *saa*$^+$	D
	HAB 14 (h)	vt_2^+ *ehxA*$^+$ *saa*$^+$	D
O113 : H21	FC 103 (f)	vt_2^+	E
	AP 97-3 (g)	vt_2^+ *ehxA*$^+$ *saa*$^+$	E
	BE 2-3 (e)	vt_2^+ *ehxA*$^+$ *saa*$^+$	F
	HT 7-14 (h)	vt_2^+	G
O117 : H7	FC 146 (f)	vt_2^+	H
	FC 149 (f)	vt_2^+	H
	FT 156 (f)	vt_2^+	I
	FT 161 (f)	vt_2^+	I
	FG 163 (f)	vt_2^+	I
	AM 214-1 (a)	vt_2^+	I
	HT 1-14 (h)	vt_2^+	I
	HT 2-2 (h)	vt_2^+	I
	AP 32-1 (g)	vt_2^+	J
O157 : H7	HT 2-15 (h)	vt_2^+ *eae*$^+$ *ehxA*$^+$	K
	FB 3 (f)	vt_2^+ *eae*$^+$ *ehxA*$^+$	K
	FB 80 (f)	vt_2^+ *eae*$^+$ *ehxA*$^+$	K
	FB 22 (f)	vt_2^+ *eae*$^+$ *ehxA*$^+$	L
	FB 81 (f)	vt_2^+ *eae*$^+$ *ehxA*$^+$	L
	FC O157 (f)	vt_2^+ *eae*$^+$ *ehxA*$^+$	M
O171 : H2	FB 27 (f)	vt_2^+	N
	FB 38 (f)	vt_2^+	N
	FB 49 (f)	vt_2^+	N
	FB 58 (f)	vt_2^+	N
	AM 174-1 (a)	vt_2^+	N
	AM 200-2 (a)	vt_2^+	N
	AM 203-3 (a)	vt_2^+	N
	AM 217-1 (a)	vt_2^+	N
	FO 140 (f)	vt_2^+	O
	CM 20-7 (m)	vt_2^+	O
	FB 8 (f)	vt_2^+	P
O174 : H21	FC 101 (f)	vt_2^+	Q
	AM 174-2 (a)	vt_2^+	Q
	AM 170-3 (a)	vt_1^+ vt_2^+ *ehxA*$^+$	Q
	T 186-3 (g)	vt_2^+	Q
	FB 10 (f)	vt_2^+	R
	FB 33 (f)	vt_2^+	R
	FO 122 (f)	vt_2^+	R
	AM 178-2 (a)	vt_2^+	R
	CM 25-12 (m)	vt_2^+	R

*Origin: g, grazing cattle; f, cattle in feedlot; h, hamburger; a, cattle at abattoir; m, minced meat; e, evisceration tray (at abattoir).

†Previously determined by monoplex PCR assays (Parma *et al.*, 2000; Padola *et al.*, 2004) and confirmed in this study by multiplex PCR (Paton & Paton, 2002).

isolates), O171 : H2 (11 isolates) and O174 : H21 (nine isolates), and are described in Table 1. All *E. coli* strains were routinely grown in Luria–Bertani (LB) broth at 37 °C and stored at −70 °C with 20 % (v/v) glycerol.

RAPD fingerprinting. Template preparation and PCR amplification with primer M13 (Birch *et al.*, 1996) were performed as described previously (Padola *et al.*, 2002), with one modification: the thermal cycler was programmed with maximum (60 °C min^{-1}) heating/cooling rates between steps. Reproducibility of the banding patterns was checked by performing the reactions at least twice using different DNA preparations.

Reaction products (12·5 µl) were analysed in a 1·8 % agarose gel with ethidium bromide. After photography, DNA fingerprints were compared visually, and each RAPD profile was defined by the presence or absence of bands at particular positions on the gel. Patterns were considered different when at least one polymorphic band could be detected.

RESULTS AND DISCUSSION

RAPD analysis with primer M13 revealed intraserotype-specific variations among VTEC isolates belonging to serotypes O20 : H19, O113 : H21, O117 : H7, O157 : H7, O171 : H2 and O174 : H21. Bands ranged from 0·4 kb to more than 1·5 kb in size.

Three RAPD profiles were detected among seven strains of serotype O20 : H19 (Fig. 1a). Profile A was shared between two strains isolated from grazing cattle. Profile B corresponded to isolates from cattle grazing on pasture, grain-fed cattle and cattle at abattoir before slaughter, and from hamburger (one of each), and profile C corresponded to an isolate from hamburger (Table 1).

Only one profile was detected among four strains of serotype O91 : H21, one isolated from grazing cattle, two from grain-fed cattle, and one from hamburger (Fig. 1b, Table 1). All these isolates shared the same virulence profile (vt_2^+ $ehxA^+$ saa^+).

Three profiles were detected among the four isolates of serotype O113 : H21 (Fig. 1c). Profile E was shared between two strains, one from one animal in a feedlot and one from an animal grazing on pasture (one from each); profile F corresponded to a strain isolated from an evisceration tray in an abattoir, and profile G to an isolate from hamburger (Table 1).

Nine isolates of serotype O117 : H7, all sharing the same virulence profile (vt_2^+), were grouped in three profiles (Fig. 1d). Two isolates from the same feedlot corresponded to profile H; three isolates from two different feedlots, one from an animal at abattoir, and two from hamburgers were all profile I, while profile J was represented by only one isolate (from an animal grazing on pasture) (Table 1). All these profiles were very similar, and only differed in one or two bands.

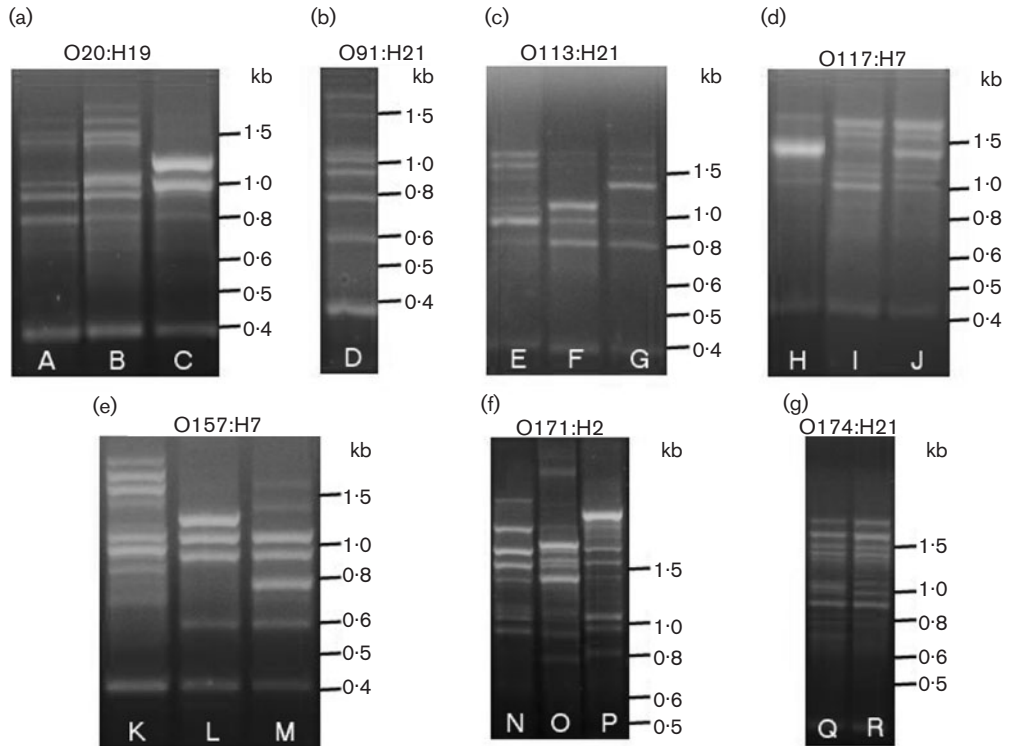

Fig. 1. RAPD profiles of VTEC isolates belonging to serotypes O20 : H19 (a), O91 : H21 (b), O113 : H21 (c), O117 : H7 (d), O157 : H7 (e), O171 : H2 (f) and O174 : H21 (g). Molecular mass (in kb) is indicated at the right of each gel.

Six isolates of serotype O157 : H7 that shared the same virulence profile (vt_2^+ eae^+ $ehxA^+$) belonged to three RAPD profiles (Fig. 1e). Three isolates corresponded to profile K (two from one feedlot and the other from hamburger), two to profile L (two isolates from the same feedlot), and a single strain from another feedlot to profile M (Table 1). Strains isolated from grain-fed cattle which belonged to profiles K and L corresponded to animals from the same feedlot.

Three RAPD profiles were found among 11 isolates of serotype O171 : H2, all of which shared the same virulence profile (vt_2^+) (Fig. 1f, Table 1). Profile N was the most common, as it was represented by eight isolates (four from animals from one feedlot and the others from animals at an abattoir). Two strains, one from a feedlot and the other from minced meat, corresponded to profile O. Profile P was represented by a single strain, isolated from a feedlot. Among strains isolated from animals in one feedlot, two similar profiles were found: profile N, which corresponded to four isolates, and profile P.

Two very similar profiles, with only a one-band difference, were present among nine isolates of serotype O174 : H21 (Fig. 1g). Four strains corresponded to profile Q (two were abattoir-collected, one was from a grain-fed animal and the other from a grazing animal). Profile R was shared by five isolates: three from two feedlots, one from an animal at an abattoir, and one from minced meat. All these isolates shared the same virulence profile (vt_2^+), except for one isolate from an animal at an abattoir which corresponded to profile Q and was vt_1^+ vt_2^+ $ehxA^+$ (Table 1). This was one of the few situations in which some strains shared the same RAPD profile although presenting a different virulence pattern.

Although some rapid PCR-based methods, such as those based on randomly amplified fragments (RAPD), or repetitive element PCR (Rep-PCR), may not give sufficient discrimination as a consequence of the highly clonal nature of VTEC O157 (Willshaw *et al.*, 2001), we were able to find three different RAPD profiles among six strains belonging to serotype O157 : H7.

Few studies have characterized non-O157 VTEC strains by RAPD fingerprinting. Kumar *et al.* (2001) characterized isolates from fish, clam and water, and Khan *et al.* (2002) studied strains isolated from human stool samples, cow stool samples, and beef samples in India; however, in both studies, strains were not serotyped. Schmidt *et al.* (1999) analysed RAPD patterns of 33 non-O157 VTEC and demonstrated three larger clusters, consisting of isolates belonging to serogroups O26, O103 and O111.

Our study compares VTEC strains belonging to several serotypes and successfully applies the RAPD technique to study the intraserotype genetic diversity among them. Some isolates, although previously regarded as homogeneous according to their virulence profile, showed different RAPD profiles. The only exception was the group of strains belonging to serotype O91 : H21, which could not be differentiated by RAPD. This could indicate clonality among strains belonging to this serotype or the inability of primer M13 to differentiate them.

Interestingly, in a preliminary study, two human O157 : H7 isolates analysed by this technique showed two new profiles, different from those found among the six O157 : H7 strains studied here (data not shown).

In conclusion, the diversity found in many serotypes may be useful for future epidemiological studies of VTEC strains, of both O157 and non-O157 serogroups, and this could also be extended to VTEC strains isolated from humans. The methodology used in this study constitutes a satisfactory tool to discriminate VTEC belonging to several serotypes, and can be applied to epidemiologically linked isolates from food and humans, making it possible to identify a chain of transmission.

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