Epidemiol. Infect. (2007), **135**, 688–694. © 2006 Cambridge University Press
doi:10.1017/S0950268806007102 Printed in the United Kingdom

Distribution of putative adhesins in Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from different sources in Chile

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(*Accepted 28 June 2006; first published online 21 August 2006*)

SUMMARY

The distribution of three putative adhesin genes in 123 Shiga toxin-producing (STEC) strains was determined by PCR. The STEC strains were isolated from human patients ($n=90$) and food ($n=33$) and were characterized by serogroup, virulence markers (*eae*, stx_1, stx_2) and adherence factors (*efa*1, $lpfA_{O157}$, *saa*) genes. Serogroups O157 (64·4%) and O26 (28·8%) were the most frequent among human strains and the majority (60·6%) of food strains were serologically non-typable. The adhesin genes *efa*1 (90%) and $lpfA_{O157}$ (73·3%) were the most common in humans strains and *saa* (45·5%) in food strains. The presence of these genes in addition to *eae* in STEC from different sources may suggest a relevant role in their pathogenesis.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are recognized as emergent pathogens that have been implicated in numerous foodborne outbreaks and enteric infections around the world [1]. These microorganisms colonize the gut and can cause watery diarrhoea, haemorrhagic colitis (HC) and haemolytic–uraemic syndrome (HUS). The most prevalent serotype associated with severe infections and HUS is *E. coli* O157:H7 [2]. Although the virulence factor best characterized in this serotype is the production of Shiga toxin (STX), adherence of strains to the gastrointestinal epithelium also plays a key role during infection [3]. The more virulent serotypes of STEC harbour a pathogenicity island termed locus of enterocyte effacement (LEE), which is associated with intimate adherence to epithelial cells, the initiation of host signal transduction pathways and with the formation of the typical attaching and effacing lesions (A/E) [4, 5]. The protein intimin is encoded by the *eae* gene which is located in the LEE locus, and this protein is the only adherence factor so far proven to be associated with intestinal colonization *in vivo* [6, 7]. The *eae* gene is present in the most virulent strains, but the isolation of disease-associated strains lacking this gene suggests the existence of other adherence factors, a number of which have been described by several investigators: Iha (IrgA homologue adhesin), associated with adherence to HeLa cells in a non-fimbriated strain [8]; Efa1 (EHEC factor for adherence), required for bacterial adherence to Chinese hamster ovary (CHO) cultured cells [9]; ToxB, required for total adherence in *E. coli* O157:H7 Sakai strain [10]; Saa (STEC autoagglutinating adhesin), described in STEC O113 LEE negative strains [11]; Sfp (sorbitol-fermenting plasmid-encoded fimbriae), fimbriae present in sorbitol-fermenting STEC O157:H$^-$ [12] and Lpf (long polar fimbriae), fimbriae of *E. coli* O157:H7 [13]. Other reports have implicated STEC proteins (Iha, Cah and OmpA), in addition to Efa1, Saa and Lpf, as mediators of adherence but their

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Table 1. *Oligonucleotides sequence of primers for PCR of STEC and adhesin gene*

Gene	Primer sequence 5′-3′	Size of product (bp)	Reference
eae	tcaatgcagttccgttatcagtt gtaaagtccgttaccccaacctg	482	[15]
stx_1	cagttaatgtggtggcgaagc caccagacaatgtaaccgctg	348	[21]
stx_2	atcctattcccgggagtttacg gcgtcatcgtatacacaggagc	584	[21]
*efa*1	aactatcctgccgcctcaga gcctgcgataacagcatcaa	456	This study
$lpfA_{O157}$	ccttgcgtactgtccgttga agcgaccagggtattgctgt	273	This study
saa	cgtgatgaacaggctattgc atggacatgcctgtggcaac	119	[22]
ERIC1	gtgaatccccaggagcttacat		[16]

Table 2. *STEC strains isolated from human and food samples*

Sample	Clinical manifestations or food source	No. of strains	Serogroup
Human samples ($n=90$)	Acute diarrhoea	57	O157 (25/57)
			O26 (26/57)
			O174 (2/57)
			O91 (1/57)
			O125 (1/57)
			ONT* (2/57)
	HC	7	O157 (7/7)
	HUS	26	O157 (26/26)
Food samples ($n=33$)	Hamburger	20	ONT* (12/20)
			O113 (7/20)
			O2 (1/20)
	Bovine meat	10	ONT* (4/10)
			O125 (2/10)
			O114 (2/10)
			O113 (1/10)
			O158 (1/10)
	Chicken	1	ONT* (1/1)
	Sausage	2	ONT* (2/2)

HC, Haemorrhagic colitis; HUS, haemolytic–uraemic syndrome.
* ONT, Non-typable with available *E. coli* antisera.

role in pathogenesis has yet to be defined [14]. We describe here the detection and distribution of these three putative adherence factors, among a collection of human and food strains of STEC.

MATERIALS AND METHODS

Bacterial strains

The STEC strains studied were isolated from 2000 to 2003 from human patients with different clinical syndromes [acute diarrhoea (57), HC (7) and HUS (26)] and from food samples [hamburger (20), bovine meat (10), chicken (1), sausage (2)]. The strains were collected by public health services from different regions of Chile, mainly from Santiago, and food strains were recovered from different meat products. Eighty-three human strains were from unique patients and seven were isolated from two patients in two separate outbreaks.

Stool samples were plated on McConkey agar and incubated at 37 °C for 24 h. For food samples, 12·5 g was mixed with 125 ml trypticase soy broth, blended in a stomacher, and 100 μl were plated on McConkey agar and incubated at 37 °C for 24 h. *E. coli* strains were identified by standard biochemical tests and serotyped by agglutination using commercial available antisera (O26, O55, O86, O111, O119, O114, O125, O126, O127, O128, O142, O157, O158) (PROBAC, Sao Paulo, Brazil); three strains of serogroups O91 and O174, were kindly serotyped at the Laboratory for Foodborne Zoonoses, Canada.

Detection of virulence genes

The presence of *eae*, stx_1 and stx_2 genes was determined by the multiplex PCR of Vidal *et al*. [15]. Strains were grown on McConkey agar overnight and five lactose-positive colonies were suspended in 150 μl of 1% Triton X-100, boiled for 10 min, and

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Table 3. stx *genotype and distribution of* eae, efa*1,* lpfA$_{O157}$ *and* saa *genes in serotypes of STEC from humans and food samples*

	No. of strains								
	Source		Positive by PCR adherence factor genes			*stx* genotype			
Serotype	H	F	*eae*	*efa*1	*lpfA*$_{O157}$	*saa*	*stx*$_1$	*stx*$_2$	*stx*$_1$–*stx*$_2$
O157 (58/123)									
	50	—	50	50	50	0	0	50	0
	2	—	2	0	2	0	0	2	0
	2	—	2	2	0	0	0	2	0
	1	—	0	0	0	0	0	1	0
	1	—	1	1	0	0	0	1	0
	1	—	0	1	1	0	0	1	0
	1	—	1	0	0	0	0	1	0
Non-O157(65/123)									
O26									
	13	—	13	13	0	0	13	0	0
	11	—	11	11	11	0	10	1	0
	2	—	2	0	0	0	0	2	0
O174	2	—	0	0	0	2	0	0	2
O91	1	—	0	0	0	1	0	1	0
O125	1	—	0	1	0	1	0	1	0
	—	1	1	0	0	1	0	1	0
	—	1	0	0	0	1	0	0	1
O2	—	1	0	0	0	1	0	1	0
O113	—	6	0	0	0	0	0	6	0
	—	1	0	0	0	1	0	1	0
O158	—	1	0	0	0	1	0	0	1
O114	—	1	0	0	0	0	0	1	0
	—	1	0	0	0	1	0	1	0
ONT*	2	2	2	2	0	0	2	0	
		10	0	0	0	0	2	8	0
		1	0	1	0	0	0	1	0
		7	0	0	0	7	0	2	5
		1	1	1	0	0	1	0	0
		1	0	1	0	1	0	1	0

H, human; F, food.
* ONT, Non-typable with available *E. coli* antisera.

centrifuged for 5 min at 8000 rpm; 3 μl of the supernatant was used as template for the PCR reaction. STEC strains EDL 933 (*eae*, *lpfA*$_{O157}$ and *efa*1) and 472-1 (*saa*) were used as positive controls in the PCR. Amplification of *eae*, *stx*$_1$, *stx*$_2$, *efa*1, *lpfA*$_{O157}$ and *saa* genes was performed with the oligonucleotides described in Table 1. The primers for *efa*1 and *lpfA*$_{O157}$ were designed from sequences available in the GenBank database using OMIGA 2.0 software (Oxford Molecular Ltd, Madison, WI, USA) for alignment and the Primer 3 program (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) for primer design. Each PCR reaction (*efa*1, *lpfA*$_{O157}$ and *saa*) was carried out independently and performed in 50 μl reaction mixture containing 1× reaction buffer (10 mM Tris–HCl, 50 mM KCl), 1·5 mM $MgCl_2$, 1 mM dNTPs, 10 pmol each primer, 0·25 U *Taq* DNA polymerase (Biotools, Madrid, Spain) and 3 μl template DNA. Samples were amplified for 35 cycles, with each cycle consisting of 1·5 min at 94 °C for denaturing, 1·5 min at 60 °C for primer annealing and 1·5 min at 72 °C for strand elongation. PCR products were visualized following electrophoresis in 1·5% agarose gels and staining with ethidium bromide; amplicons were identified by reference to molecular size markers.

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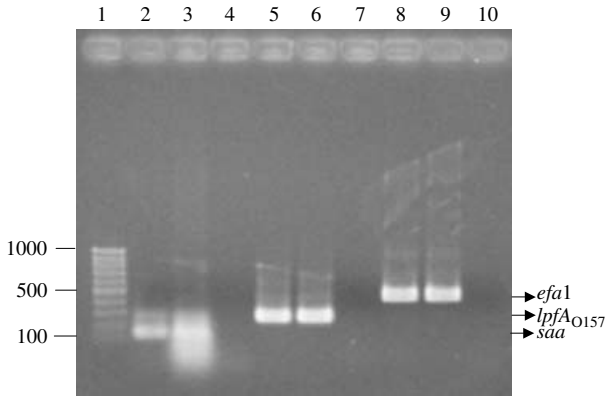

Fig. 1. PCR analysis of *saa*, $lpfA_{O157}$ and *efa*1 genes in controls and samples strains. Lane 1, ladder 100 bp; lane 2, STEC E026-00; lane 3, STEC 472-1; lane 4; negative control for *saa* gene; lane 5, EHEC E030-00; lane 6, EHEC EDL 933; lane 7, negative control for $lpfA_{O157}$ gene; lane 8, EHEC E030-00; lane 9, EHEC EDL 933; lane 10, negative control for *efa*1 gene.

DNA fingerprinting of strains

Strains were typed by indexing variation in the conserved Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences of the genome. DNA was extracted from overnight growth of pure subcultures and cells were lysed by boiling for 10 min. Genotyping was performed using the ERIC1 fingerprinting assay [16]. PCR amplifications were performed in 25 μl volumes containing 2·5 mM $MgCl_2$, 2 U *Taq* DNA polymerase (Promega, Madison, WI, USA), 1 mM dNTPs, 1·5 μl template DNA and 100 pmol ERIC1 primer. ERIC-PCR products were visualized following electrophoresis in 3% agarose gels and stained as above. Electrophoretic patterns were entered into Treecon for Windows v. 1.3b matrix analysis software (University of Konstanz, Germany).

Statistical test

Statistical analyses were performed with the SPSS 11.0.1. package for Windows (SPSS Inc., Chicago, IL, USA). The χ^2 test was used for calculations and P values of $<0{\cdot}05$ indicated a significant difference in the distribution of genes in different serogroups.

RESULTS

For human samples, 58 (64·4%) strains belonged to the O157 serogroup and 26 (28·8%) were of the O26 serogroup. These two serogroups accounted for 25 and 26 of the 57 cases of acute diarrhoea and all of the HC and HUS cases were caused by serogroup O157. The remainder of the human strains fell into serogroups O174, O91, O125 and two strains were serologically non-typable (ONT). In contrast, 19 of the 33 food strains were ONT and, with the exception of O125, none of the serogroups identified in food strains was found in human strains (Table 2).

Table 3 shows that all *E. coli* serogroup O157 strains harboured only the stx_2 gene. The majority (23/26) serogroup O26 isolates were positive for the stx_1 gene while both genes were present in the two strains of serogroup O174 tested and in two other serogroups (O125 and O158) and five ONT strains. Gene stx_1 was not detected in any of the other serotypable isolates but was present in three ONT strains. On the other hand the stx_2 gene occurred in varying numbers of five other serogroups and in 14 ONT strains.

The amplification of *saa*, $lpfA_{O157}$ and *efa*1 genes produced PCR products of 129, 273 and 456 bp respectively (Fig. 1). The *eae* gene was detected in 84 (93·3%) human strains compared with 2/33 of food strains. The most prevalent putative adhesin genes in human samples were *efa*1 (90%), and $lpfA_{O157}$ (74·4%), while the *saa* gene was present in only six (6·6%) of these strains; *efa*1 gene in 79 of the *eae*-positive strains and $lpfA_{O157}$ in 65 strains. The most prevalent gene among food strains was *saa* (45·5%), while *efa*1 was present in only three strains (9%). The $lpfA_{O157}$ gene was not detected.

By ERIC-PCR profiles the 122 STEC strains were grouped into two main clusters, which could be further subdivided into two minor clusters each (Fig. 2). Cluster Ia comprised the great majority of serogroup O157 strains while Ib mainly contained food strains of serogroup O113. A small number of food strains fell in cluster IIa but IIb consisted mainly of disease-associated serogroup O26 strains and 10 strains of other serogroups including six representatives of serogroup O157 from HC and HUS cases. These groupings did not correlate with the adhesin genes profile.

DISCUSSION

We determined by specific PCR assays the distribution of three putative adhesin genes in STEC strains isolated from human and food sources, belonging to different serogroups and isolated over a period of 4 years. We found that *eae* was the most

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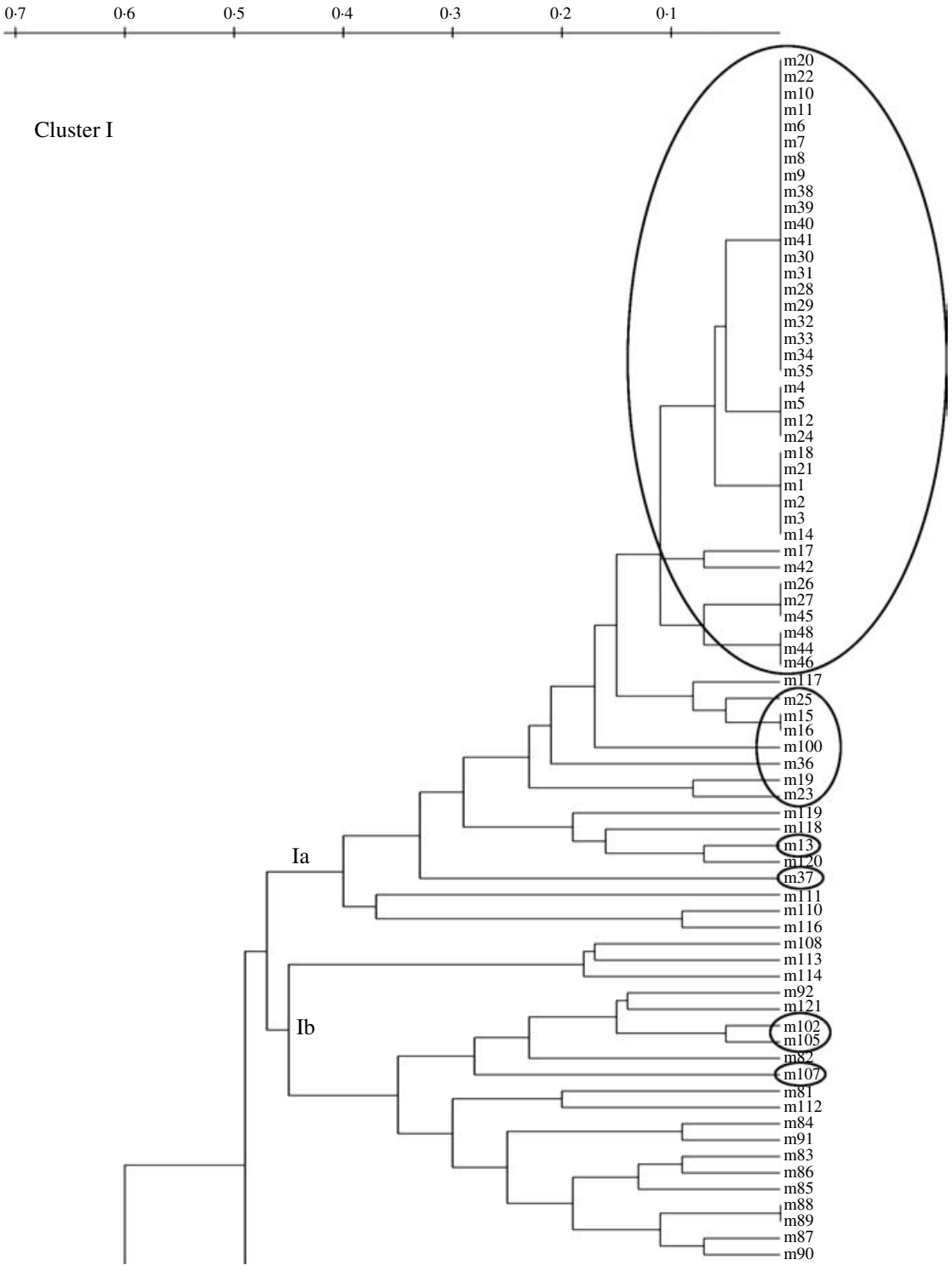

Fig. 2. Dendrogram comparing ERIC-CR profiles of STEC strains isolated from human and food sources. Human strains are encircled.

prevalent gene in human STEC strains (93%) and there was a close association between the *eae*-positive strains and the presence of *efa*1 ($P=0{\cdot}0006$) and $lpfA_{O157}$ ($P=0{\cdot}005$) genes, a situation similar to that described by Toma *et al*. [17]. The majority of human strains corresponded to O157 and O26 serogroups, which have not been previously associated with the presence of the *saa* gene [11]; we only detected this

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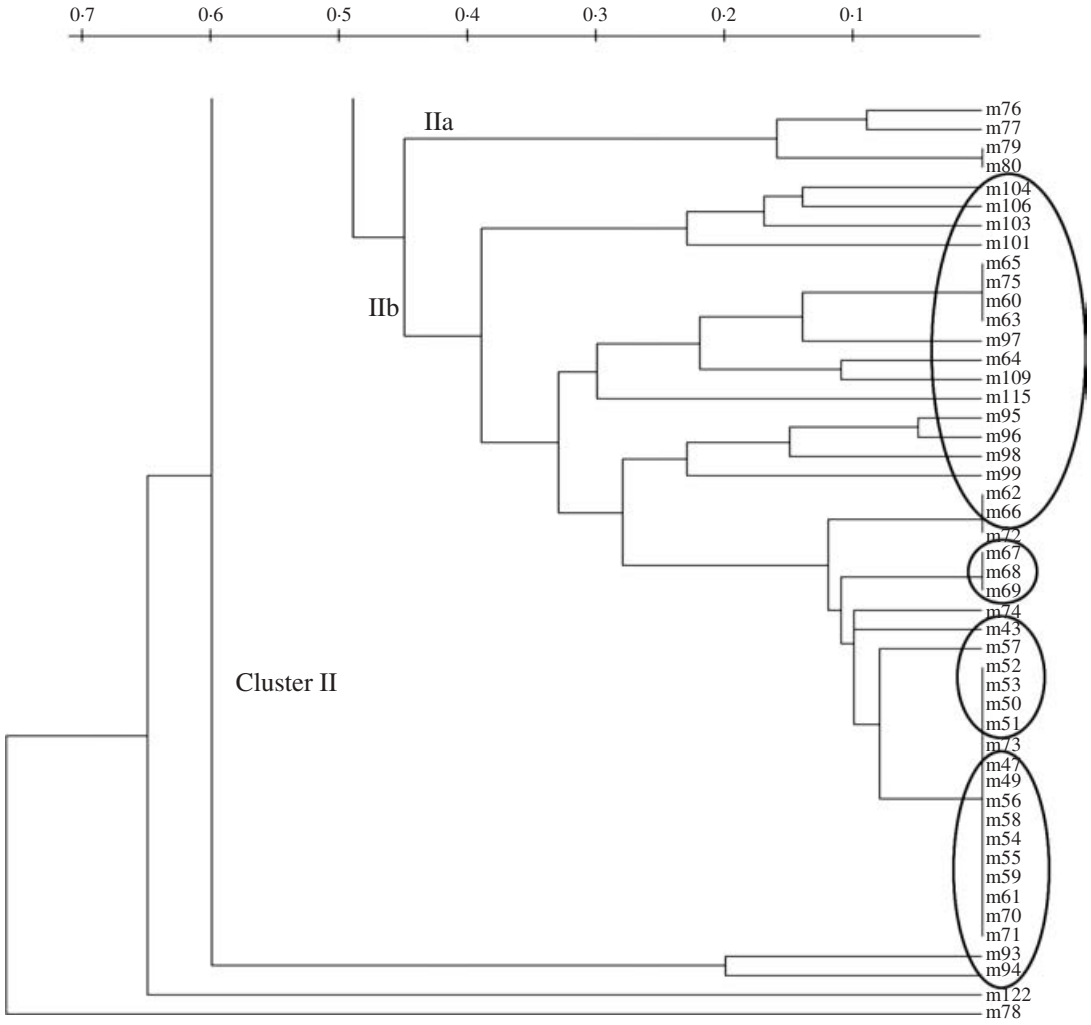

Fig. 2. (*cont.*)

gene in four (6%) human isolates belonging to O125, O174 and O91 serogroups.

The *eae* gene was very rare in food strains while the *saa* gene was the most common marker identified in these strains confirming the negative correlation of the presence the two genes previously observed by Paton *et al*. [11]. Other studies on the frequency of the *saa* gene indicate that it is more frequently found in bovine STEC [18, 19]. Overall, these results suggest that the Saa protein may have a more important role in attachment of STEC organisms to the bovine gut than the human intestine [18].

Our data indicate that Chilean STEC strains of human origin belong to different serogroups than those common in food with the exception of serogroup O125 which was found in both groups of samples. It is noteworthy that some strains isolated only from food, e.g. serogroups O2 and O113 have been associated with human disease previously [20]. Other serogroups in food such as O114 and O158 could represent normal intestinal microbiota of animals or are serogroups not yet described as human pathogens. The most prevalent serogroups of human strains were O157 and O26 which were entirely absent from foodstuffs. In conclusion, the distribution of the adherence genes of STEC was related to the source of isolation. In human strains the *eae* gene predominated along with the putative adherence factors *efa*1 and $lpfA_{O157}$ and, therefore, these gene products may be potential candidates for vaccines directed to inhibit the colonization of the human intestine.

ACKNOWLEDGMENTS

We thank Dr Alfredo Torres for critical reading of the manuscript. This work was supported by Doctoral Fellowship, Conicyt AT-403131 and Grant DI INC 03/04, Universidad de Chile.

DECLARATION OF INTEREST

None.

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