RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY (2006) 9:53-60
www.im.microbios.org

INTERNATIONAL MICROBIOLOGY

Miguel Blanco[1]
Leonel Lazo[2]
Jesús E. Blanco[1]
Ghizlane Dahbi[1]
Azucena Mora[1]
Cecilia López[1]
Enrique A. González [1¶]
Jorge Blanco[1*]

Serotypes, virulence genes, and PFGE patterns of enteropathogenic *Escherichia coli* isolated from Cuban pigs with diarrhea

[1]*E. coli* Reference Laboratory (LREC), Department of Microbiology and Parasitology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain
[2]Faculty of Veterinary, Central University Marta Abreu de Las Villas, Cuba

Received 27 November 2005
Accepted 10 January 2006

*Corresponding author:
J. Blanco
Laboratorio de Referencia de *E. coli* (LREC)
Departamento de Microbiología y Parasitología
Facultad de Veterinaria
Universidad de Santiago de Compostela
Campus de Lugo
27002 Lugo, España
Tel/Fax: +34-982285936
E-mail: jba@lugo.usc.es

¶Deceased

Summary. Thirty-six enteropathogenic *Escherichia coli* strains isolated from Cuban pigs with diarrhea were serotyped and screened by PCR for the presence of virulence genes. The 36 isolates belonged to 11 O serogroups and 14 O:H serotypes, with 53% of the isolates belonging to only two serotypes: O141:H$^-$ (13 isolates) and O157:H19 (6 isolates). Genes coding for STb, STa, VT2e, and LT toxins were identified in 69, 61, 53, and 6% of the isolates, respectively. The most prevalent fimbrial adhesin was F18, detected in 22 (61%) isolates. The gene encoding F6 (P987) colonization factor was identified in three (8%) isolates. None of the 36 isolates assayed contained genes encoding F4 (K88), F5 (K99), or F41. The seropathotype O141:H$^-$:STa/STb/VT2e/F18 (13 isolates) was the most frequently detected, followed by O157:H19:VT2e/F18 (5 isolates). A genetic diversity study, carried out by pulsed-field gel electrophoresis (PFGE) of 24 representative isolates, revealed 21 distinct restriction patterns clustered in 18 groups (I–XVIII). Isolates of the same serotype were placed together in a dendrogram, but isolates of serotype O157:H19 showed a high degree of polymorphism. The results of this study demonstrate the presence in Cuba of different clusters among one of the most prevalent serotypes isolated from pigs with diarrhea. Further experiments are needed to determine whether some of these clusters have appeared recently; if so, their evolution, as well as their possible association with pathogenicity in farms should be studied. [**Int Microbiol** 2006; 9(1):53-60]

Key words: *Escherichia coli* · enteropathogenic *E. coli*· enterotoxins · ETEC · PFGE · porcine diarrhea · STEC · VTEC

Introduction

Escherichia coli is an important cause of diarrhea in newborn and postweaning pigs, and is responsible for significant losses in large-scale farms worldwide. Enterotoxigenic (ETEC) and verotoxigenic (VTEC) *E. coli* are the main categories of diarrheagenic *E. coli* that cause enteric infections in pigs [4,10,12,17,18,20,27,30]. Porcine ETEC strains produce two major classes of enterotoxins: heat-labile toxin (LT), a high-molecular-weight toxin, and heat-stable toxins (STa and STb, also named STI and STII), which are small, poorly immunogenic proteins [4,18,30]. Porcine VTEC strains, also called Shiga toxin-producing *E. coli* (STEC) [7], produce the edema verotoxin (VTe), also named VT2 variant (VT2v) or Shiga-toxin 2e (Stx2e) [24–26]. Some *E. coli* strains, mainly those isolated from pigs with postweaning diarrhea (PWD) and edema disease, can produce both enterotoxins and VT2e

toxin, and are appropriately referred to as ETEC/VTEC [5,33]. *E. coli* that produce VT2e in the absence of enterotoxins cause edema disease but not diarrhea [24,25].

Many porcine ETEC and VTEC strains have fimbrial structures on their surface that, like LT, STa, and STb enterotoxins, are usually plasmid mediated. These fimbriae are termed colonization antigens and they enable the bacteria to colonize the epithelial surface of the porcine small intestine [5,15,17,31]. F4 (K88) and F18 [8,12,29,31] are fimbriae that have been implicated in PWD. But, whereas the F18 fimbria is associated almost exclusively with PWD, the F4 (K88) fimbria is also the main colonization factor implicated in neonatal diarrhea (ND) [3,30]. Other types of fimbriae, including F5 (K99), F6 (P987), and F41, are usually found on porcine ETEC isolated from newborn pigs with diarrhea [9,15]. The majority of porcine ETEC and VTEC strains belong to a limited range of O serogroups. The main O groups that occur in different parts of the world are O8, O64, O138, O139, O141, O147, O149, and O157 [5,12,14,17,33, 38,41]. However, the distribution and frequencies of serogroups can vary considerably from region to region and over time in a given region. The objective of this study was to identify the serotypes, virulence factors, and genetic diversity of ETEC and VTEC isolated from Cuban pigs with diarrhea.

Materials and methods

***E. coli* isolates**. Thirty-six *Escherichia coli* strains isolated from pigs with diarrhea were investigated in this study. Fecal samples were collected from July to Dec. 2002 from seven (A–G) piggeries located in the provinces of Villa Clara and Cienfuegos (central region of Cuba). Pigs were divided into two age groups: younger than 30 days (piglets with neonatal diarrhea, ND) and older than 30 days (pigs with PWD). The fecal samples were plated onto MacConkey agar (Oxoid, UK), and *E. coli* isolates were identified by standard biochemical procedures(API-20E, bioMérieux, France).

Serotyping. O and H antigens were determined according to the method described by Guinée et al. [17] and employing all available O (O1–O185) and H (H1–H56) antisera in the *E. coli* Reference laboratory (LREC) at the University of Santiago de Compostela, Lugo, Spain: [http://www.lugo.usc.es/ecoli]. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove nonspecific agglutinins.

Table 1. Primer sequences used to amplify target genes coding for virulence factors, and predicted lengths of PCR amplification products

Target gene	Primer	Oligonucleotide sequence (5´→3´)	Fragment size (bp)	Ref.
LT-I	LT-Ia LT-Ib	GGCGACAGATTATACCGTGC CCGAATTCTGTTATATATGTC	708	[5]
LT-II	LT-II-1 LT-II-2	AGATATAATGATGGATATGTATC TAACCCTCGAAATAAATCTC	300	[39]
STa	STA-1 STA-2	ATTTTTATTTCTGTATTGTCTTT GGATTACAACACAGTTCACAGCAGT	176	[39]
STb	STb-1 STb-2	ATCGCATTTCTTCTTGCATC GGGCGCCAAAGCATGCTCC	175	[5]
VT1	VT1-A VT1-B	CGCTGAATGTCATTCGCTCTGC CGTGGTATAGCTACTGTCACC	302	[6]
VT2	VT2-A VT2-B	CTTCGGTATCCTATTCCCGG CTGCTGTGACAGTGACAAAACGC	516	[6]
VT2e	VT2e-A VT2e-B	CCTTAACTAAAAGGAATATA CTGGTGGTGTATGATTAATA	230	[5]
F4 (K88)	AM005 AM006	GGTGATTTCAATGGTTCGGTC ATTGCTACGTTCAGCGGAGCGC	773	[11]
F5 (K99)	K99-A K99-B	CCAGCGCCCGGCAGTAATGACTGC CCACCATTAGACGGAGCGCGG	278	This study
F6 (P987)	P987-A P987-B	GCGCCCGCTGAAAACAACACCAGC GTACCGGCCGTAACTCCACCG	467	This study
F18	F18-A F18-B	GGTACTGTTGCACCAAGCGG CGACGCCTTAACCTCCTGCCCC	225	This study
F41	F41-A F41-B	GGCTATGGAAGACTGGAGAGGG GGGGTGACTGAGGTCATCCC	551	This study

The primers used to amplify VT1 (Stx1) and VT2 (Stx2) genes were able to detect VT1, VT2, and the variants: VT1c (Stx1c), VT2c (Stx2c), VT2d (Stx2d) and VT2e (Stx2e).

Table 2. Serotypes O:H, virulence genes, and pulsed-field gel electrophoresis (PFGE) patterns of *Escherichia coli* isolates

Serotype	No. (origina/farm)	LT	STa	STb	VT2e	F18	P987	PFGE *XbaI* pattern
O7:H15	1 (PWD/A)	+	+	+				XVI
O15:H45	1 (ND/A)			+				
O15:H45	1 (PWD/C)			+				
O15:H$^-$	1 (ND/A)			+				V
O20:H$^-$	1 (ND/A)		+					XVII
O35:H6	2 (ND/C)			+		+		
O45:H9	1 (PWD/E)			+				II
O64:H$^-$	1 (ND/D)		+				+	III
O64.H$^-$	1 (ND/E)		+	+				XVIII
O84:H$^-$	1 (ND/D)		+	+				XIV
O141:H$^-$	4 (PWD/B)		+	+	+	+		IX
O141:H$^-$	7 (PWD/B)		+	+	+	+		
O141:H$^-$	1 (PWD/B)		+	+	+	+		XI
O141:H$^-$	1 (PWD/E)		+	+	+	+		X
O149:H23	1 (ND/D)	+	+	+				XV
O157:H19	2 (PWD/C)				+	+		VIII
O157:H19	1 (ND/F)				+	+		VI
O157:H19	2 (PWD/E)				+	+		VI
O157:H19	1 (ND/B)			+				VII
O169:H38	1 (PWD/A)		+					I
ONT:H19	1 (PWD/C)				+	+		IV
ONT:H19	1 (PWD/C)			+		+		
ONT:H$^-$	1 (ND/B)		+				+	XII
ONT:H$^-$	1 (ND/B)		+				+	XIII

[a]Origin: ND, neonatal diarrhea (from the first few days of birth to weaning); PWD, postweaning diarrhea.

The O antisera were produced in the LREC and the H antisera were obtained from the Statens Serum Institut (Copenhagen, Denmark).

Detection of virulence genes by PCR. Toxins (LT, STa, STb, VT1, VT2, and VT2e) and adhesins (F4/K88, F5/K99, F6/P987, F18, and F41) were detected using PCR, as described by Blanco et al. [5–7] and Penteado et al. [39]. The base sequences and predicted sizes of the amplified products for the specific oligonucleotide primers are shown in Table 1.

Pulsed-field gel electrophoresis. PFGE was carried out using a Chef Mapper system (Bio-Rad, Hemel Hempstead, United Kingdom) at 14ºC in 0.5´ Tris/Borate/EDTA (TBE) electrophoresis buffer following the Enternet-proposed standard-protocol for PFGE [http://www.foodborne-net.de/content/e25/e70/e580/index_ger.html]. Agarose-embedded DNA cleaved with the restriction enzyme *Xba*I (0.2–0.8 U/ml) (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Run times and pulse times were 2.20–54.0 s for 22 h with linear ramping. PFGE was used to establish genetic similarity among a representative group of 24 strains, including the following serotypes: O7:H15 (1 strain), O15:H$^-$ (1), O20:H$^-$ (1), O45:H9 (1), O64:H$^-$ (2), O84:H$^-$ (1), O141:H$^-$ (6), O149:H23 (1), O157:H19 (6), O169:H38 (1), ONT:H$^-$ (2), ONT:H19 (1). The PFGE pulsotypes were compared by analyzing TIFF files with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis of the Dice similarity indices, based on the unweighted pair group method using arithmetic averages (UPGMA), was carried out to generate a dendrogram describing the relationship among pulsotypes. The criterion for discriminating between isolates was a difference in the patterns of at least one restriction fragment.

Results

Serogroups and serotypes. Thirty-six strains of *E. coli* isolated from Cuban pigs with diarrhea were serotyped. These 36 isolates belonged to 11 O serogroups and 14 O:H serotypes, with 53% of the isolates belonging to only two serotypes: O141:H$^-$ (13 isolates) and O157:H19 (6 isolates) (Table 2).

Toxin genes. PCR amplification of the toxin genes showed that 100% of the isolates had genes encoding at least one of the four toxins (LT, STa, STb, and VT2e) studied. The gene encoding STb enterotoxin (25 isolates) was the most prevalent, followed by STa (22 isolates), VT2e (19 isolates), and LT (2 isolates) (Table 2). All 36 isolates were negatives for toxins: VT1 (Stx1), VT2 (Stx2), VT1c (Stx1c), VT2c (Stx2c), and VT2d (Stx2d).

Adhesin genes. PCR analysis of the 36 *E. coli* isolates showed that 25 (69%) carried at least one fimbrial gene. The most prevalent fimbrial adhesin was F18, detected in 22

(61%) isolates. The gene encoding F6 (P987) colonization factor was identified in three (8%) isolates (Table 2). None of the 36 isolates assayed contained genes encoding F4 (K88), F5 (K99), or F41.

Seropathotypes. Although the 36 porcine *E. coli* isolates belonged to 17 different seropathotypes (associations between serotypes and virulence genes), only two accounted for 50% of isolates. Seropathotype O141:H⁻:STa/STb/VT2e/F18 (13 isolates) was the most common, followed by O157:H19:VT2e/F18 (5 isolates) (Table 2).

Macrorestriction fragment analysis by PFGE. Genetic diversity was analyzed in 24 representative isolates by PFGE, which revealed 21 distinct restriction patterns, based on a difference of a single band as a basis for discrimination between isolates. In the dendrogram produced by the UPGMA algorithm, the isolates were clustered in 18 groups (I–XVIII; 1–4 strains per group) of 85% similarity according to the Dice similarity index. Isolates of the same seropathotype were grouped together in the dendrogram, but a high degree of polymorphism among isolates of serotype O157:H19 was observed. In fact, analysis of the six strains of this serotype revealed six different restriction patterns, clustered in three groups (VI, VII, VIII) of 85% similarity. The highest homogeneity was observed among seropathotype O141:H⁻ STa STb VT2e F18, with four strains showing 100% similarity (group IX) (Fig. 1).

Discussion

Diarrhea is one of the most common diseases of newborn and postweaning pigs worldwide. It is widely accepted that specific serotypes and pathotypes of ETEC and VTEC strains are responsible for most ND and PWD in pigs [5,12,13,18,20]. In the present study, although the 36 isolates belonged to 14 different O:H serotypes, more than half of the ETEC and VTEC strains belonged to only two serotypes: O141:H⁻ and O157:H19. ETEC and VTEC strains of these two serotypes, especially serotype O141:H⁻, have also been frequently detected in pigs from other countries [12,31,37,40].

We found that STb was the most prevalent toxin, accounting for 69.5% of the 36 *E. coli* strains isolated from Cuban piglets with diarrhea. Our findings are in accordance with those of other studies [5,12,21,27,34,44]. The high preva-

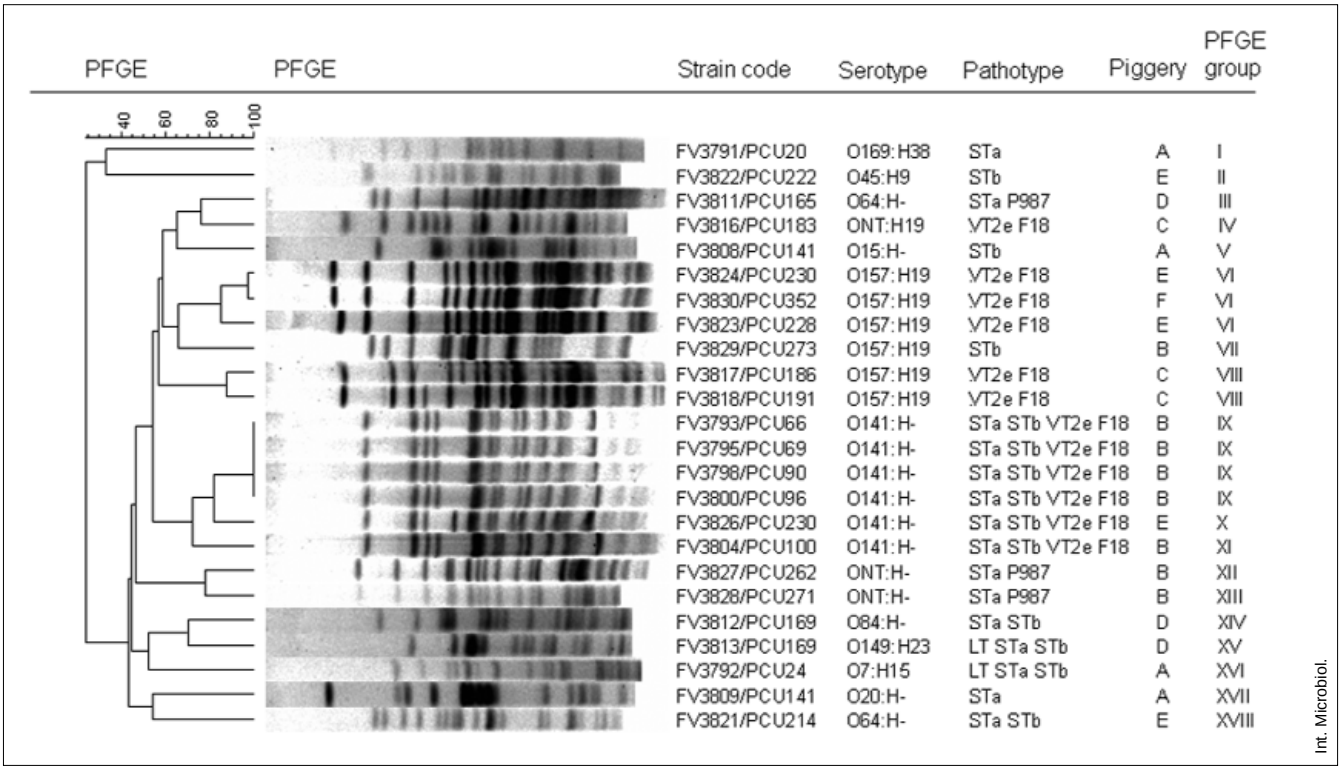

Fig. 1. Dendrogram, generated by Bionumeric software, showing distances calculated by the Dice similarity index of PFGE XbaI patterns among 24 *Escherichia coli* strains. The degree of similarity (%) is shown on the scale.

lence of the gene coding for STb strongly implicates this toxin in swine diarrhea. VT2e produced by VTEC plays an important role in the pathogenesis of edema disease [26]. One target of the VT2e toxin is the endothelial cells of small blood vessels, resulting in edema at specific locations. Macleod et al. [24,25] demonstrated that VT2e is not involved in the onset of diarrhea in pigs. F18-positive *E. coli* producing-VT2e in the absence of enterotoxins cause edema disease, but not diarrhea. In our study, 19 (52.7%) of 36 isolates harbored the VT2e gene; in 13 of them, VT2e occurred in combination with STa and STb enterotoxins. Note that most VT2e-positive isolates identified in the current study belonged to only two serotypes (O141:H$^-$. and O157:H19). In previous studies, VT2e production in porcine strains was mainly associated with serotypes O138:H14, O138:H$^-$, O139:H1, O141:H4, O147:H6, and O157:H19 [5,12,14,16, 31,37,40].

Historically, F4 (K88) colonization antigen has been incriminated as the predominant fimbrial type associated with ND and PWD in pigs. However, the prevalence of F18 strains in PWD and edema disease is higher than that of F4 (K88) [8,12,31]. Using PCR analysis, we showed that 19 (82.6%) of the 23 *E. coli* strains isolated from PWD carried genes coding for F18 colonization antigen compared to only three (23.0%) of the 13 *E. coli* strains isolated from ND. These results are in accordance with those of Ojeniyi et al. [34] and Frydendahl [12], who, respectively, reported the detection of F18 in 34 and 39% of *E. coli* strains isolated from PWD in Denmark. A survey conducted in the Czech Republic by Alexa et al. [2] also detected 312 strains (40%) positive for F18 in the 772 *E. coli* strains isolated from weaned piglets with diarrhea. Hide et al. [22] demonstrated that, among 480 hemolytic *E. coli* strains from diarrheic weaned piglets, a significant percentage (62%) carried the F18 gene. Also, Wittig et al. [44] showed the presence of F18 in 75% of 380 *E. coli* isolated from the intestinal contents of diarrheic weaned piglets. By contrast, in some countries the prevalences of F18 is low. For example, in Korea, Kwon et al. [23] found that only 19% of the 230 *E. coli* strains isolated from PWD were positive for the F18 gene, and in Poland, Osek [36] detected only 10 (3%) F18-positive strains among 372 PWD *E. coli* isolated from piglets.

In Australia, Hide et al. [22] observed that F18-expressing strains of *E. coli*, isolated from weaned pigs, belonged to serogroups O8, O45, O138, O141, and O157. Alexa et al. [2] and Nagy et al. [31] showed that the predominant serogroups of most Czech and Hungarian F18-expressing ETEC and VTEC strains were O138, O139, O141, and O157. In accordance with these results, we identified the F18 gene in strains of serogroups O35, O141, and O157. Regarding the association of F18 with toxins, Cuban *E. coli* isolates positive for the F18 gene carried also STa, together with STb and VT2e toxins, VT2e only, or STb only (see Table 2).

F4 (K88) and F18 have been implicated as fimbriae in PWD [8,12,29,31]. However, whereas the F18 fimbria is associated almost exclusively with PWD, the F4 (K88) fimbria is the main colonization factor implicated in ND [5,30,33,34,41,42]. Surprisingly, in the current study, we did not detect any F4 (K88)-positive strains. However, we did find that ETEC positive for F4 (K88) fimbria were the most prevalent among *E. coli* strains isolated in Slovakia from neonatal porcine diarrhea (detected in 38%) and PWD (detected in 19%) (Blanco et al. unpublished data), and obtained similar results in Spain (Blanco et al. unpublished data). Alexa et al. [2], Ojeniyi et al. [34], Osek [36], and Wittig et al. [44] reported prevalences of F4 (K88) strains originating from PWD in the Czech republic (19%), Denmark (26%,) Poland (19%), and Germany (14%). In the United States, a study led by Wilson and Francis [42] reported that 71% of PWD strains carried the F4 (K88) fimbrial antigen. Similarly, Nagy et al. [28] reported that 61% of 205 Hungarian *E. coli* isolates derived from fatal cases of PWD or edema disease of piglets were positive for F4 (K88) fimbria. By contrast, in Japan and in Korea, the prevalence of F4 (K88) strains isolated from piglets with PWD is low [23, 32]. Several studies demonstrated that O8, O149, and O157 were the O serogroups most frequently associated with the fimbria F4 (K88) [5,12,21,34,42]. In Spain, O149:H10:LT/STb and O157:H$^-$:LT/STb are the most prevalent seropathotypes detected among F4 (K88) ETEC strains [5] (Blanco et al. unpublished data).

According to Dean et al. [9], infection with ETEC strains carrying F6 (P987) fimbria is age-related. In several countries, F6 (P987) is the most frequently detected fimbria among strains isolated from suckling piglets with diarrhea [15,42]. Our results confirm these observations, as we found the F6 (P987) gene in three (25%) of 12 *E. coli* strains isolated from ND but in none of the 26 *E. coli* strains collected from postweaning pigs.

In the present study, *E. coli* strains with genes encoding F5 (K99) or F41 fimbrial colonization antigens were not detected. The prevalence of ETEC strains expressing these two fimbriae also varies with geographic locations. High prevalences of F5 (K99) and F41 fimbriae were found in Sweden [41], Canada [21], Germany [43], and Spain [15] from ND, whereas in Poland and Slovakia F5- (K99) and F41-producing *E. coli* have been isolated only rarely from piglets with diarrhea [35] (Blanco et al. unpublished data).

Only a few studies have reported the genetic relatedness of *E. coli* strains isolated from pigs with diarrhea [1,19,

31,37]. Hampson et al. [19] applied multilocus enzyme electrophoresis to analyze the genetic diversity of 79 Australian *E. coli* isolates from pigs with PWD (serogroups O8, O138, O141, O149, and O157), and 18 isolates of serotype O149:K91:F4(K88) from unweaned pigs from Australia, Indonesia, and Denmark. These were divided into 57 electrophoretic types (ETs). The authors reported a considerable genetic heterogeneity among PWD isolates of the same serogroup. They found high genetic diversity among isolates of serogroups O8 and O138, while most strains of serogroups O141 and O149 were more closely related. Nagy et al. [31] also applied multilocus enzyme electrophoresis to analyze 43 Hungarian *E. coli* isolates from weaned pigs suffering either from edema disease or from diarrhea. The 43 strains, which belonged to O5, O21, O109, O138, O139, O147, O141, O157, and OX serogroups, showed 18 distinct ETs based upon genetic variation in 20 enzyme loci. Strong genetic homogeneity was detected among O157 and O138 isolates, while the other serogroups showed considerable genetic diversity, especially O139 strains. Osek [37] used PFGE to analyze 82 *E. coli* strains, isolated from pigs with PWD from geographically separated farms in the western part of Poland. The 82 strains, which belonged to four serogroups (O138, O139, O141, and O149), showed 13 different PFGE patterns. Although a high degree of polymorphism among different serotypes was observed, strains belonging to the same serological group were closely related. In fact, the 25 isolates of serotype O149:K91 generated only two PFGE types. In contrast to the results obtained by Osek [37], we observed 36 distinct PFGE restriction patterns in 46 ETEC and VTEC isolates from Slovakia that were obtained from pigs with PWD (Blanco et al. unpublished data). Although isolates of the same serotype were placed together in the dendrogram, a high degree of polymorphism was detected among certain serotypes. In fact, 13 distinct PFGE patterns resulted from 15 O149:H10 isolates analyzed, although 14 of those 15 isolates carried the same virulence genes (LT, STb, F4/K88). Similar results were obtained in Spain among isolates of the most prevalent serotype (O157:H$^-$ LT STb F4/K88) (Blanco et al. unpublished data). Supporting our previous results, the genetic diversity observed in the present study with 24 representative porcine ETEC and VTEC isolates revealed 21 distinct PFGE restriction patterns clustered in 18 groups. Isolates of the same seropathotype were placed together in the dendrogram, which showed a high degree of polymorphism among isolates of serotype O157:H19 (Fig. 1). We even detected different PFGE groups among O141:H$^-$ strains with the same pathotype (Sta STb VT2e F18) from the same farm (farm B; groups IX and XI). This very interesting finding confirms the presence in Cuba of different clusters among one of the most prevalent serotypes isolated from pigs with diarrhea. More isolates should be studied to determine whether some of these clusters have appeared recently; if so, their evolution, as well a possible relationship to pathogenicity in farms should be studied.

In conclusion, our results indicate that in Cuba, as in other countries, ETEC and VTEC isolates from pigs with diarrhea belong to a restricted number of serogroups and serotypes. Apparently, two seropathotypes are predominant in PWD: O141:H$^-$:STa/STb/VT2e/F18 and O157:H19:VT2e/F18. Nevertheless, as the number of *E. coli* isolates characterized in the current study is small, further studies, involving more farms and geographic areas, are necessary before definitive conclusions can be reached regarding the most important seropathotypes implicated in porcine diarrhea in Cuba. The results obtained in our study, however, have special relevance for the design and development of *E. coli* vaccines against enteric infections in pigs to be administered in Cuban piggeries.

Acknowledgments. This paper is dedicated to the memory of Dr. Enrique A. González (1956–2005), eminent scientist, excellent professor of microbiology, and very good friend. We thank Monserrat Lamela for her skillful technical assistance. This work was supported by a grant from the Spanish Fondo de Investigación Sanitaria (FIS G03-025-COLIRED-O157), and two grants from the Xunta de Galicia (PGIDIT02BTF26101PR and PGIDIT04RAG261014PR).

References

1. Aarestrup FM, Jorsal SE, Ahrens P, Jensen NE, Meyling A (1997) Molecular characterization of *Escherichia coli* strains isolated from pigs with edema disease. J Clin Microbiol 35:20-24
2. Alexa P, Salajka E, Hamrik J, Nejezchleb A (1995) Pathogenic strains of *Escherichia coli* in weaned piglets in the Czech Republic. Pig News Inform 16:81N-84N
3. Alexa P, Stouracová K, Hamrík J, Salajka E (2002) Gene typing of the colonisation factors F18 of *Escherichia coli* isolated from piglets suffering from post-weaning oedema disease. Vet Med–Czech 47:132-136
4. Blanco J, Blanco M, Garabal JI, González EA (1991) Enterotoxins, colonization factors and serotypes of enterotoxigenic *Escherichia coli* from humans and animals. Microbiol SEM 7:57-72
5. Blanco M, Blanco JE, Gonzalez EA, Mora A, Jansen W, Gomes TAT, Zerbini LF, Yano T, Pestana de Castro AF, Blanco J (1997) Genes coding for enterotoxins and verotoxins in porcine *Escherichia coli* strains belonging to different O:K:H serotypes: relationship with toxic phenotyes. J Clin Microbiol 35:2958-2963
6. Blanco M, Blanco JE, Mora A, Dhabi G, Alonso MP, González EA, Bernárdez MI, Blanco J (2004) Serotypes, virulence genes, and intimin types of Shiga toxin (Verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (*eae*-ξ). J Clin Microbiol 42:645-651
7. Blanco M, Podola NL, Krüger A, Sanz ME, Blanco JE, González EA, Dahbi G, Mora A, Bernárdez MI, Etcheverría AI, Arroyo GH, Lucchesi PMA, Parma AE, Blanco J (2004) Virulence genes and intimin types of

Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina. Int Microbiol 7:269-276
8. Cheng DX, Gao S, Jiao X, Liu XF (2004) Prevalence of serogroups and virulence factors of *Escherichia coli* strains isolated from pigs with postweaning diarrhoea in eastern China. Vet Microbiol 103:13-20
9. Dean EA, Whipp SC, Moon HW (1989) Age-specific colonization of porcine intestinal epithelium by 987P-pilated enterotoxigenic *Escherichia coli.* Infect Immun 57:82-87
10. Francis DH (2002) Enterotoxigenic *Escherichia coli* infection in pigs and its diagnosis. J Swine Health Prod 10:171-175
11. Franklin MA, Francis DH, Baker D, Mathew AG (1996) A PCR-based method of detection and differentiation of K88 adhesive *Escherichia coli*. J Vet Diagn Invest 8:460-463
12. Frydendahl K (2002) Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet Microbiol 85:169-182
13. Garabal JI Gonzalez EA, Vazquez F, Blanco J, Blanco M (1995) Toxigenic *Escherichia coli* in Spanish piggeries from 1986 to 1991. Vet Microbiol 47:17-25
14. Garabal JI Gonzalez EA, Vazquez F, Blanco J, Blanco M, Blanco JE (1996) Serogroups of *Escherichia coli* isolated from piglets in Spain. Vet Microbiol 48:113-123
15. Garabal JI, Vázquez F, Blanco J, Blanco M, González EA (1997) Colonization antigens of enterotoxigenic *Escherichia coli* isolated from piglets in Spain. Vet Microbiol 54:321-328
16. González EA, Blanco J (1985) Production of cytotoxin VT in enteropathogenic and non-enteropathogenic *Escherichia coli* strains of porcine origin. FEMS Microbiol Lett 26:127-130
17. Guinée PAM, Jansen WH, Wadström T, Sellwood R (1981) *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In: Leew PW, Guinée PAM (ed) Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea: Current Topics in Veterinary and Animal Science 13, Martinus-Nijhoff, The Hague, Netherlands, pp 126-162
18. Gyles CL (1994) *Escherichia coli* enterotoxins. In Gyles CL (ed) *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, UK, pp 337-364
19. Hampson, DJ, Woodwar JM, Connaughton ID (1993) Genetic analysis of porcine postweaning diarrhea. Epidemiol Infect 110:575-581
20. Hampson, DJ (1994) Post-weaning *E. coli* diarrhoea in pigs. In Gyles CL (ed) *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, UK, pp 171-191
21. Harel J, Lapointe H, Fallara A, Lortie LA, Bigras-Poulin M, Larivière S, Fairbrother JM (1991) Detection of genes for fimbrial antigens and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with diarrhea. J Clin Microbiol 29:745-752
22. Hide EJ, Connaughton ID, Driesen SJ, Hasse D, Monckton RP, Sammons NG (1995) The prevalence of F107 fimbriae and their association with Shiga-like toxin II in *Escherichia coli* strains from weaned Australian pigs. Vet Microbiol 47:235-243
23. Kwon D, Choi C, Jung T, Chung HK, Kim JP, Bae SS, Cho WS, Kim J, Chae C (2002) Genotypic prevalence of the fimbrial adhesins (F4, F5, F6, F41 and F18) and toxins (LT, Sta, STb, and Stx2e) in *Escherichia coli* isolated from postweaning pigs with diarrhoea or oedema disease in Korea. Vet Rec 12:35-37
24. MacLeod DL, Gyles CL, Valdivieso-Garcia A, Clarke RC (1991) Physicochemical and biochemical properties of purified *Escherichia coli* Shiga-like toxin II variant. Infect Immun 59:1300-1306
25. MacLeod DL, Gyles CL, Wilcock BP (1991) Reproduction of edema disease of swine with purified Shiga-like toxin-II variant. Vet Pathol 28:66-73
26. Marquers LRM, Peiris JSM, Cryz SJ, O'Brien AD (1987) *Escherichia coli* strains isolated from pigs with edema disease produce a variant of Shiga-like toxin II. FEMS Microbiol Lett 44:33-38
27. Moon WH, Schneider RA, Moseley SL (1986) Comparative prevalence of four enterotoxin genes among *Escherichia coli* isolated from swine. Am J Vet Res 47:210-212
28. Nagy B, Casey TA, Moon HW (1990) Phenotype and genotype of *Escherichia coli* isolated from pigs with postweaning diarrhea in Hungary. J Clin Microbiol 28:651-653
29. Nagy B, Whipp SC, Imberechts H, Bertschinger HU, Dean-Nystrom EA, Casey TA, Salajka E (1997) Biological relationship between F18ab and F18ac fimbriae of enterotoxigenic and verotoxigenic *Escherichia coli* from weaned pigs with oedema disease or diarrhoea. Microb Pathog 22:1-11
30. Nagy B, Fekete PZ (1999) Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. Vet Res 30:259-284
31. Nagy B, Wilson RA, Whittam TS (1999) Genetic diversity among *Escherichia coli* isolates carrying *f18* genes from pigs with porcine postweaning diarrhea and edema disease. J Clin Microbiol 37:1642-1645
32. Nakazawa M, Sugimoto C, Isayama Y, Kashiwazaki M (1987) Virulence factors in *Escherichia coli* isolated from piglets with neonatal and post-weaning diarrhea in Japan. Vet Microbiol 13:291-300
33. Noamani BN, Fairbrother JM, Gyles CL (2003) Virulence genes of O149 enterotoxigenic *Escherichia coli* from outbreaks of postweaning diarrhea in pigs. Vet Microbiol 97:87-101
34. Ojeniyi B, Ahrens P, Meyling A (1994) Detection of fimbrial and toxin genes in *Escherichia coli* and their prevalence in piglets with diarrohea. The application of colony hybridization assay, polymerase chain reaction and phenotypic assays. Zentralbl Vetereninarmed B 41:49-59
35. Osek J, Gallien P, Truszczynski M, Protz D (1999) The use of polymerase chain reaction for determination of virulence factors of *Escherichia coli* isolated from pigs in Poland. Comp Immunol Microbiol Infect Dis 22:163-174
36. Osek J (199) Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. Vet Microbiol 68:209-217
37. Osek J (2000) Clonal analysis of *Escherichia coli* strains isolated from pigs with post-weaning diarrhea by pulsed-field gel electrophoresis. FEMS Microbiol Lett 186:327-331
38. Parma AE, Sanz ME, Viñas MR, Cicuta ME, Blanco JE, Boehringer SI, Vena MM, Roibon WR, Benitez MC, Blanco J, Blanco M (2000) Toxigenic *Escherichia coli* isolated from pigs in Argentina. Vet Microbiol 72:269-276
39. Penteado AS, Ugrinovich LA, Blanco J, Blanco M, Blanco JE, Mora A, Andrade JRC, Corrêa SS, Pestana de Castro, AF (2002) Serobiotypes and virulence genes of *Escherichia coli* strains isolated from diarrheic and healthy rabbits in Brazil. Vet Microbiol 89:41-51
40. Prager R, Bauerfeind R, Tietze E, Behrend J, Fruth A, Tschape H (2004) Prevalence and deletion types of the pathogenicity island ETT2 among *Escherichia coli* strains from oedema disease and colibacillosis in pigs. Vet Microbiol 99:287-294
41. Söderlind O, Thafvelin B, Möllby R (1988) Virulence factors in *Escherichia coli* strains isolated from Swedish piglets with diarrhea. J Clin Microbiol 26:879-884
42. Wilson R.A, Francis DH (1986) Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. Am J Vet Res 47:213-217
43. Wittig W, Fabricius C (1992) *Escherichia coli* types isolated from porcine *E. coli* infections in Saxony from 1963 to 1990. Zentralbl Bakteriol 277:389-402
44. Wittig W, Klie H, Gallien P, Lehmann S, Timm M, Tschape P (1995) Prevalence of the fimbrial antigens F18 and K88 and of enterotoxins and verotoxins among *Escherichia coli* isolated from weaned pigs. Zentral Bakteriol 283:95-104

Serotipos, genes de virulencia y patrones de PFGE de *Escherichia coli* enteropatógenos aislados de cerdos con diarrea en Cuba

Resumen. En este estudio, 36 cepas enteropatógenas de *Escherichia coli* aisladas de cerdos con diarrea en Cuba fueron serotipadas y sometidas a un cribado mediante PCR para detectar la presencia de genes de virulencia. Los 36 aislamientos pertenecían a 11 serogrupos O y 14 serotipos O:H. No obstante, el 53% de los aislamientos presentaron solamente dos serotipos: O141:H$^-$ (13 aislamientos) y O157:H19 (6 aislamientos). Los genes que codifican las toxinas STb, STa, VT2e y LT fueron identificados, respectivamente, en 69%, 61%, 53%, y 6% de los aislamientos. La adhesina fimbrial predominante fue la F18, que se detectó en 22 (61%) de los aislamientos. El gen que codifica el factor de colonización F6 (P987) fue identificado en tres (8%) aislamientos. Ninguno de los 36 aislamientos ensayados presentaba los genes que codifican las adhesinas F4 (K88), F5 (K99) y F41. El seropatotipo O141:H$^-$:STa/STb/VT2e/F18 fue el detectado con más frecuencia (13 aislamientos), seguido del O157:H19:VT2e/F18 (5 aislamientos). El estudio de la diversidad genética, que se llevó a cabo mediante electroforesis en gel en campo pulsado (PFGE) en 24 aislamientos representativos, reveló 21 patrones de restricción diferentes repartidos en 18 grupos (I-XVIII). Los aislamientos del mismo serotipo se agruparon en un dendograma, pero los aislamientos del serotipo O157:H19 mostraron un alto grado de polimorfismo. Los resultados de este estudio indican que en Cuba existen diferentes complejos génicos (*clusters*) en uno de los serotipos predominantes aislados de cerdos afectados de diarrea. Son necesarios más estudios para saber si algunos de estos complejos han aparecido recientemente y, en ese caso, para poder analizar su evolución y determinar la posible relación con su poder patógeno en las granjas. [**Int Microbiol** 2006; 9(1):53-60].

Palabras clave: *Escherichia coli* · *E. coli* enteropatógena · enterotoxinas · ETEC · PFGE · diarrea porcina · STEC · VTEC

Sorotipos, genes de virulência e patrões de PFGE de *Escherichia coli* enteropatogênica isoladas de porcos com diarréia em Cuba

Resumo. Neste estudo, 36 cepas enteropatógenas de *Escherichia coli* isoladas de porcos com diarréia em Cuba foram sorotipadas e submetidas a uma sondagem por PCR para detectar a presença de genes de virulência. Os 36 isolamentos pertenciam a 11 sorogrupos Ou e 14 sorotipos Ou:H. Não obstante, 53% dos isolamentos presentaram somente dois serotipos: Ou141:H$^-$ (13 isolamentos) e Ou157:H19 (6 isolamentos). Os genes que codificam as toxinas STb, STA, VT2e e LT foram identificados, respectivamente, em 69%, 61%, 53%, e 6% dos isolados. A adesina fimbrial predominante foi o F18, que se detectou em 22 (61%) dos isolados. O gene que codifica o fator de colonização F6 (P987) foi identificado em três (8%) isolados. Nenhum dos 36 isolamentos testados apresentou os genes que codificam as adhesinas F4 (K88), F5 (K99) e F41. O soropatotipo O141:H$^-$:STA/STb/VT2e/F18 foi o detectado com mais freqüência (13 isolamentos), seguido do O157:H19:VT2e/F18 (5 isolamentos). O estudo da diversidade genética, que foi realizado mediante electroforese em gel de campo pulsado (PFGE) em 24 isolados representativos, revelou 21 patrões de restrição diferentes repartidos em 18 grupos (I-XVIII). Os isolamentos do mesmo sorotipo foram agrupados juntos em um dendograma, mas os isolamentos do sorotipo O157:H19 mostrou um alto grau de polimorfismo. Os resultados deste estudo indicam que em Cuba existem diferentes complexos genéticos (*clusters*) em um dos sorotipos predominantes isolados de porcos afetados por diarréia. São necessários mais estudos a fim de se comprovar se destes complexos apareceram recentemente e, nesse caso, poder analisar sua evolução e determinar a possível relação com seu poder patógeno nas fazendas. [**Int Microbiol** 2006; 9(1):53-60]

Palavras chave: *Escherichia coli* · *E. coli* enteropatógena · enterotoxinas · ETEC · PFGE · diarréia porcina · STEC · VTEC