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Genetic Diversity among *Escherichia coli* Isolates Carrying *f18* Genes from Pigs with Porcine Postweaning Diarrhea and Edema Disease

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Multilocus enzyme electrophoresis was applied to detect allelic variation and multilocus genotypes (electrophoretic types [ETs]) among 43 *Escherichia coli* isolates from weaned pigs suffering from edema disease or from diarrhea. ETs were analyzed in relation to O serogroups and virulence genes (*sta*, *stb*, *lt*, stx_2, and *f18*) by DNA hybridization. Genomic diversity was the lowest in serogroup O138, while virulence genes (stx_2 and *f18*) were the most uniform in serogroup O139. In general, the serogroups or toxin and F18 fimbria types were not related to selected ETs, suggesting that the toxin and *f18* fimbria genes in *E. coli* isolates from pigs with postweaning diarrhea or edema disease occur in a variety of chromosomal backgrounds.

Major factors influencing bacterial pathogenicity are the families of attachment factors (comprised of fimbriae) and toxins often encoded on plasmid DNA, thereby moving horizontally among the population of *Escherichia coli*. It has been clearly demonstrated that the adherence factors and toxins of enterotoxigenic *E. coli* that infect farm animals, namely, calves and pigs, occur in isolates from a limited number of serotypes (13, 15, 16, 25, 29), suggesting host specificity of adhesin-receptor interactions and genetic links between determinants of O types and adhesin and toxin types. It has been shown that porcine postweaning diarrhea (PWD) strains often carry enterotoxin genes and express F4 (and occasionally also F5, F6, or F41) fimbriae (29), whereas edema disease (ED) isolates tend to be F4, F5, F6, and F41 negative and strains often produce Shiga-like toxin 2v (2, 9, 14), detectable on Vero cells, also called verotoxigenic *E. coli*. The application of multilocus enzyme electrophoresis (MLEE) to the study of *E. coli* populations has demonstrated that the O serogroups associated with infectious diseases occur in a variety of chromosomal backgrounds (21).

The purpose of the present study was to assess the diversity of chromosomal backgrounds and to characterize the distribution of virulence factors of PWD and ED strains of *E. coli* lacking the fimbriae described above. To this end, we characterized a variety of porcine PWD and ED strains by MLEE, DNA probe assays, and serological tests. In addition to testing for toxigenicity, attention was directed to the newly described fimbriae F18ab (F107) and F18ac (2134P and 8813), adhesins recently associated with ED and PWD isolates as described by Bertschinger et al. (3), Casey et al. (4), Nagy et al. (18), and Salajka et al. (23) respectively, and proven to be variants of F18 fimbriae (10, 19, 22).

A total of 43 *E. coli* isolates were obtained from the small-intestinal contents or the feces of weaned pigs suffering from PWD or ED and were selected on the basis of producing no K88 (F4), K99 (F5), 987P (F6), or F41 fimbriae. Strains with four-digit number designations (except strain 2228) were isolated in Hungary and were partially characterized earlier (17), whereas strains with six-digit number designations were isolated in the United States and represent a subset of strains from an earlier study (30). Each strain was isolated from a different pig except for strains 2155 and 2156. Strain 2228 is a Swiss isolate, kindly provided by A. O'Brien (Bethesda, Md.). The strains were serotyped by standard methods at the Statens Seruminstitut, Copenhagen, Denmark, or at the *E. coli* Reference Center, Pennsylvania State University, University Park, Pa. The presence of F18ac fimbriae was analyzed by fluorescent microscopy (18) using monoclonal antibodies as described previously (5) for testing bacteria grown on Iso-Sensitest agar (Oxoid, Basingstoke, United Kingdom) with alizarin yellow (Fluka AG, Buchs, Switzerland) added, in a 5% CO_2 atmosphere as described by Wittig et al. (31). Enterotoxin genes *sta*, *stb*, and *lt* as well as Shiga-like toxin (stx_2) and fimbrial (*f18*) genes were detected by colony hybridizations performed according to the method of Grunstein and Hogness (6). The *stb* probe was prepared from the recombinant plasmid pRAS-1, the *sta* probe was prepared from the recombinant plasmid pSTP6, and the *lt* probe was prepared from the recombinant plasmid pWD299. The *f18* probe that detects both F18ab and F18ac (8, 10, 22) was obtained from strain 4748, and the stx_2 probe that detects both Stx2 and Stx2v toxin genes was obtained from the recombinant plasmid pNN111-19 (20) by the method of Lee et al. (12). The probes were labeled by nick translation with [^{32}P]dATP. MLEE was applied to detect allelic variation at 20 enzyme loci by the methods described by Whittam et al. (28). Strains were characterized by their multilocus array of alleles and classified into distinct electrophoretic types (ETs). The genetic relationships among isolates were assessed by groupings inferred from cluster analysis (24, 28).

The 43 strains were allotted to serogroups O157, O147, O141, O139, O138, O109, O21, O5, and OX. The association of the serogroups with the virulence genes *sta*, *stb*, *lt*, stx_2 and *f18* and the ETs is provided in Table 1. The *f18* fimbria gene was detected in all major serogroups. Although most serogroups represented closely related ETs (Fig. 1), not all mem-

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TABLE 1. Characteristics of *E. coli* isolates from pigs with PWD and ED

Strain	Serogroup[a]	Characteristic[d]						
		sta[b]	*stb*[b]	*lt*[b]	*stx*$_2$[b]	F18ac[c]	*f18*[b]	ET
2134P	O157	+	+	−	−	+	+	11
2152	O157	+	+	−	−	+	+	11
2188	O157	+	+	−	−	+	+	11
802575	O157	+	−	+	−	−	−	18
2211	O147	+	+	−	+	+	+	1
2144	O147	+	+	−	+	+	+	1
2173	O147	+	+	−	−	+	+	4
2157	O147	+	+	−	−	+	+	16
2185	O141	+	+	−	+/−	+	+	17
2172	O141	+	+	−	−	+	+	17
2171	O141	+	+	−	−	+	+	11
2155	O141	−	+	+	−	−	+	17
2180	O141	+	+	−	−	+	+	1
2181	O141	+	+	−	−	+	+	3
2183	O141	−	−	−	+	−	+	17
820727	O141	+	−	−	−	−	−	13
2156	O141	+	+	−	−	+	+	17
2142	O139	−	−	−	+	−	+	5
2200	O139	−	−	−	+	−	+	8
2162	O139	−	−	−	+	−	+	5
2141	O139	−	−	−	+	−	+	6
2206	O139	−	−	−	+	−	+	7
2228	O139	−	−	−	+	−	+	6
750158	O139	−	−	−	−	−	−	12
881284	O139	−	−	−	+	−	+	9
911456	O139	−	−	−	+	−	+	10
2203	O138	+	+	−	+	+	+	1
750162	O138	+	+	−	−	−	+	2
750324	O138	+	+	−	+	−	+	2
750408	O138	+	+	−	+	−	+	2
750421	O138	−	+	−	+	−	−	2
760385	O138	−	−	−	−	−	+	2
760386	O138	−	+	−	+	−	−	2
831086	O138	−	−	−	−	−	−	14
890652	O138	+	+	−	+	+	+	2
2189	O109	+	+	−	−	−	−	17
2159	O21	−	+	−	−	+	+	1
2168	O5	−	−	−	+	−	−	15
2170	OX[e]	+	+	+	−	−	+	1
2192	OX	+	+	−	−	+	+	1
2193	OX	+	+	−	−	+	+	1
2209	OX	+	+	−	−	−	+	17
2207	OX	+	+	−	−	+	+	17

[a] All except two of the O139 Hungarian strains were from pigs with ED.
[b] Detected by use of a gene probe.
[c] Detected by use of a monoclonal antibody.
[d] +, present; −, absent; +/−, equivocal result.
[e] OX, not typeable with standard O antisera.

bers of a serogroup shared the same virulence genes nor did they express the same surface fimbriae.

Among the four serogroup O157 strains evaluated, three had identical phenotypes, e.g., each carried *sta* and *stb* enterotoxin and *f18* genes, while the other strain 802575, was positive for *sta* and *lt* genes and lacked the *f18* gene (Table 1). Interestingly, strain 802575 belongs to clone ET18, which is not related (genetic distance, $D = 0.580$) to the ET that defines the other three O157 isolates namely ET11 (Fig. 1). The nine O141 isolates displayed similar toxin and fimbria patterns. Seven strains carried *sta* or *stb*, all except one carried *f18*, and one strain (strain 2155) was positive for *lt* and *stb* genes. However, this serogroup (O141) was associated with five distinct clones, ET1, ET3, ET11, ET13, and ET17, which were represented in three clusters, A, C, and D (Fig. 1). Eight of nine O139 strains reacted with the *f18* gene probe, whereas none expressed the F18ac fimbria. Only one strain failed to react with the *stx*$_2$ probe, and none were positive for enterotoxin genes. In contrast to O141, the O139 strains represented by ET5, ET6, ET7, ET8, ET9, and ET10 formed a tight cluster related at a *D* of 0.1, except for strain 750158 (ET12), which is related at a *D* of 0.45 (Fig. 1). Six of nine O138 strains were positive for the *f18* gene. Six of nine, five of nine, and seven of nine isolates reacted with *stx*$_2$, *sta*, and *stb* gene probes, respectively. With the exception of one isolate, strain 831086, the serogroup O138 strains comprised a tight cluster, cluster A, containing ET1 and ET2 that are related at a *D* of 0.05. Importantly, strain 831086 was negative for all virulence factors tested in reference to cluster A strains that carry *sta*, *stb*, *stx*$_2$, and *f18* genes (Fig. 1 and Table 1). Serogroups infrequently associated with PWD, namely, O109, O21, and O5, and serogroup OX were represented by three clonal types, ET1, ET15, and ET17. These clonal types were represented among the serogroups most commonly associated with ED and PWD. The majority of this group of isolates was positive for the heat-stable toxin genes; only one strain carried the heat-labile toxin gene, and six of eight were positive for the *f18* gene (Fig. 1).

Enteric disease in the neonatal and preweaned pig is often associated with *E. coli* strains that express STa, STb, LT toxins, and K88 (F4), K99 (F5), 987P (F6), or F41 fimbria virulence factors. Recently, the identified adhesins F107 (F18ab) and 2134P (F18ac), as well as a Shiga toxin variant (Stx2v), have been associated with isolates from pigs with ED and PWD (2, 5, 8, 9, 11, 17, 18). The two adhesin factors F18ab and F18ac have been found most frequently associated with serogroups O138, O139, O141, O147, and O157 (19, 22).

Through the application of MLEE to the study of *E. coli* population genetics, Ochman et al. (21) have shown that animal *E. coli* strains of the same serotype can be represented in diverse genetic backgrounds. On the other hand, Whittam et al. (27) provided strong genetic evidence that *E. coli* O157:H7, the causative agent of hemorrhagic colitis and hemolytic uremic syndrome belongs to a pathogenic clone that occurs throughout North America. Furthermore, we have previously shown that the O157:H7 isolates are related at the serogroup level to the O157 serogroup of pigs but they are not closely related genetically (26).

The genetic diversity of *E. coli* strains isolated from pigs with PWD (overwhelmingly enterotoxigenic and 39% F4$^+$) in Australia has been recently reported by Hampson et al. (7). Using MLEE, they found 57 different ETs among 79 isolates of serogroups O8, O138, O141, O149, and O157. Cluster analysis resulted in 14 subclusters at a *D* of 0.2 that showed that only a few isolates in the four main serogroups were closely related. It was surprising that the diversity of serogroup O138 was very high for these Australian isolates (7). Their data suggested that strains causing PWD represent a variety of *E. coli* strains that may carry virulence factors associated with disease of older pigs. However, they did not test for virulence factors other than K88 (F4).

In our study focusing on F18$^+$ *E. coli* and virulence genes, we found 18 distinct ETs among the 43 isolates based upon genetic variation at 20 enzyme loci. There was a strong genetic homogeneity in serogroups O157 and O138, whereas the other serogroups evaluated were comprised of diverse ETs, with a *D* of >0.2 between them.

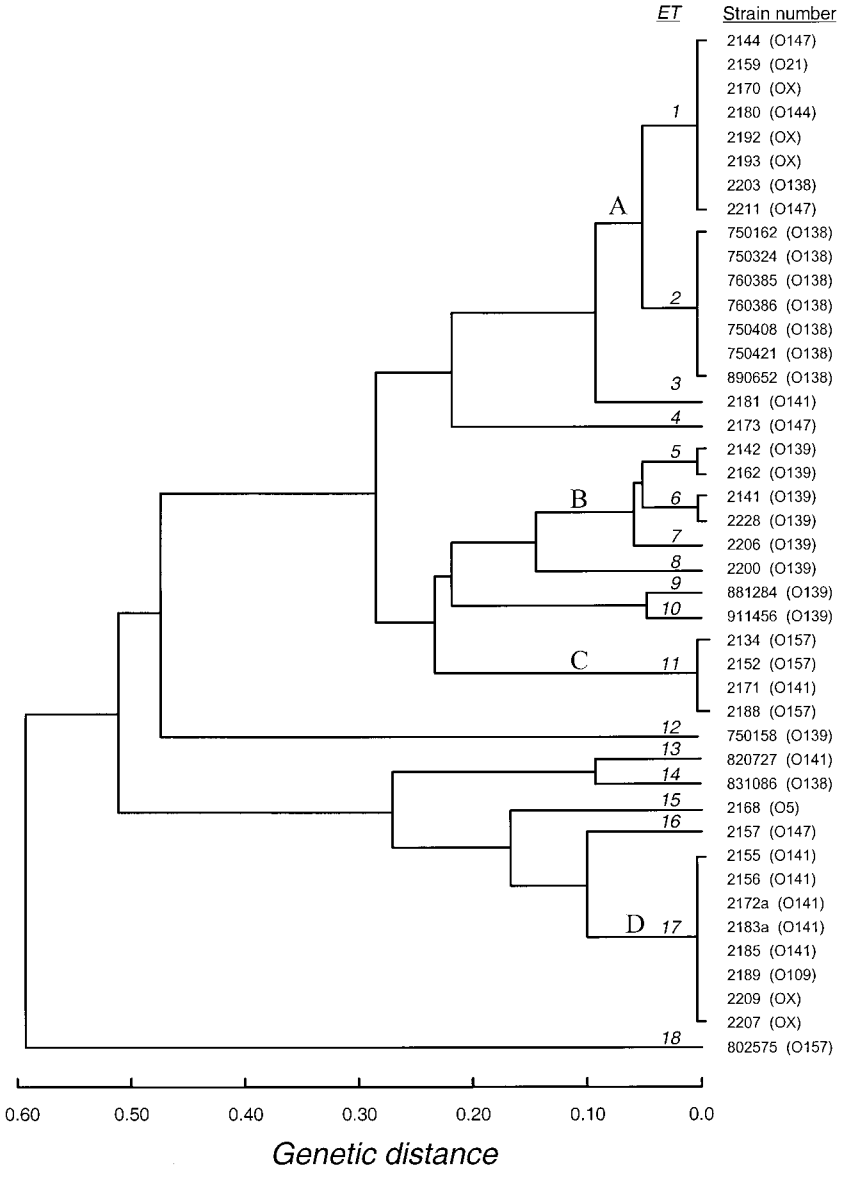

FIG. 1. Genetic relationships of 43 ETs of porcine strains isolated from pigs with PWD or ED. The dendrogram was generated by average-linkage cluster analysis, using genetic distance matrix based on the numbers of allelic mismatches between ETs.

Aarestrup et al. (1) used ribotyping and pulsed-field gel electrophoresis profiling to show how ED strains (O139, F18, and Stx2e) from different countries were related. Based on pulsed-field gel electrophoresis profiles, the Danish isolates were closely related and different from isolates from other countries (Switzerland, Hungary, Iceland, Norway, and Sweden), suggesting an association of Danish strains with the movement of animals between breeding and finishing facilities. In our study, most of the O139 strains from Hungary and the United States formed a relatively tight cluster (including seven clones), and had the most uniform virulence genes (*f18* and stx_2), consistent with the findings of the Danish study (1). Several of our ED and PWD strains were recently investigated for F18 and Stx phenotypes and showed patterns similar to the phenotypes of the Danish strains (19).

Bertschinger et al. (3) have shown that inheritance of susceptibility to intestinal colonization with F107 (F18ab) fimbriae appears to be a single recessive trait. It has also been shown that F18ab and F18ac most likely share the same receptors (19, 22). Pathogenic *E. coli* with these fimbriae would more likely be isolated from a select group of pigs and, theoretically, could represent a genetically more homogeneous group of strains. However, our data indicate the existence of a diverse population of $f18^+$ *E. coli* isolates with similar serotypes associated with PWD and ED. In our study, only eight of the strains were neither phenotype F18ac positive nor *f18* gene positive.

The strains selected for the present study represent a subset of strains from our earlier study (30). In the previous study, we tested 325 *E. coli* strains selected from the Reference Center that had been isolated from pigs diagnosed with PWD or ED in the United States and in Hungary by using MLEE as in the present study. We have shown that the serotypes associated with diarrhea (PWD) have very diverse genetic backgrounds

even though the serotypes appear to be limited and that among the four major serogroups the fimbriae and toxins in general are not related to selected genotypes. Our earlier observations (30) concur with the data reported here, that the toxin and *f18* fimbria genes of PWD strains occur in a variety of genetic backgrounds and suggest that such strains have converged by selection or recombination to these major phenotypes. In contrast, *f18*$^+$ ED strains seem to be characterized by somewhat more genetic homogeneity.

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