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Putative Adhesins of Enteropathogenic and Enterohemorrhagic *Escherichia coli* of Serogroup O26 Isolated from Humans and Cattle[▽]

Marjorie Bardiau,* Sabrina Labrozzo, and Jacques G. Mainil

Department of Infectious and Parasitic Diseases—Bacteriology, Veterinary Faculty, University of Liège, Liège B4000, Belgium

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Enterohemorrhagic *Escherichia coli* (EHEC) strains are responsible for food poisoning in developed countries via consumption of vegetal and animal food sources contaminated by ruminant feces, and some strains (O26, O111, and O118 serogroups) are also responsible for diarrhea in young calves. The prevalence of 27 putative adhesins of EHEC and of bovine necrotoxigenic *E. coli* (NTEC) was studied with a collection of 43 bovine and 29 human enteropathogenic (EPEC) and EHEC strains and 5 non-EPEC/non-EHEC (1 bovine and 4 human) O26 strains, using specific PCRs. Four "groups" of adhesins exist, including adhesins present in all O26 strains, adhesins present in most O26 strains, adhesins present in a few O26 strains, and adhesins not present in O26 strains. The common profile of EHEC/EPEC strains was characterized by the presence of *loc3*, *loc5*, *loc7*, *loc11*, *loc14*, *paa*, *efa1*, *iha*, $lpfA_{O26}$, and $lpfA_{O113}$ genes and the absence of *loc1*, *loc2*, *loc6*, *loc12*, *loc13*, *saa*, and *eibG* genes. Except for the $lpfA_{O26}$ gene, which was marginally associated with bovine EHEC/EPEC strains in comparison with human strains ($P = 0.012$), none of the results significantly differentiated bovine strains from human strains. One adhesin gene (*ldaE*) was statistically ($P < 0.01$) associated with O26 EHEC/EPEC strains isolated from diarrheic calves in comparison with strains isolated from healthy calves. *ldaE*-positive strains could therefore represent a subgroup possessing the specific property of producing diarrhea in young calves. This is the first time that the distribution of putative adhesins has been described for such a large collection of EHEC/EPEC O26 strains isolated from both humans and cattle.

Enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* strains represent two important classes of enteric pathogens that cause diarrhea in humans and animals. They have in common the ability to produce a histopathological lesion on enterocytes, called an "attaching and effacing" lesion. The intimate attachment of the bacteria to enterocytes and the localized effacement of microvilli are the main characteristics of the attaching and effacing lesion (26).

EPEC strains are an important cause of infant diarrhea in developing countries and are often associated with high mortality rates (8). Human EPEC strains are subdivided into classical (type 1) and nonclassical (type 2) strains on the basis of the production of bundle-forming pili or the presence of the encoding genes. Nonclassical EPEC strains are also present in different animal species. In bovines, nonclassical EPEC strains are associated with diarrhea in young calves of up to 3 months of age (9).

EHEC strains are considered to have evolved from EPEC strains through the acquisition of bacteriophages encoding Shiga toxins (Stxs) (31, 45). EHEC strains cause several clinical syndromes in humans (mainly in children and elderly people), such as diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. These have been responsible for large outbreaks in many developed countries, especially Japan, the United States, and the United Kingdom (26). Transmission can occur via consumption of vegetal and animal foodstuffs contaminated by ruminant feces (mainly cattle) (7). Some EHEC strains are also responsible for undifferentiated diarrhea in young calves of up to 3 months of age (24).

EPEC and EHEC strains can belong to more than 1,000 O:H serotypes. In EHEC infections, O157:H7 is the main serotype responsible for several outbreaks and sporadic cases of hemorrhagic colitis and hemolytic-uremic syndrome, but non-O157 serogroups (such as O26, O145, O111, and O103) can also be associated frequently with severe illness in humans (5, 35). Though most, if not all, EHEC serogroups are carried by healthy animal ruminants, a few are associated with diarrhea in calves (O5, O26, O111, O118, etc.). Human and animal EPEC strains also belong to a series of O antigenic groups, including O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 (6). Thus, several serogroups are present in both pathotypes (EHEC and EPEC) and can infect both humans and cattle. Although classical EPEC strains have always been regarded as host specific, EHEC strains have not, and the actual situation regarding nonclassical EPEC strains remains unknown.

The first step in EPEC and EHEC infection is the initial adherence of bacteria to intestinal cells. This adherence step could be the basis for any host specificity via the production of colonization factors, such as the bundle-forming pilus adhesin of classical human EPEC strains.

Low et al. analyzed 14 putative fimbrial gene clusters revealed by the EHEC O157:H7 Sakai sequence (21). Of these 14 putative fimbriae, several had already been described under other names, including LpfA1 (42), LpfA2 (43), F9 (20), type 1 fimbriae (32) (34), and curli fimbriae (30). Long polar fimbria (Lpf)-encoding genes had also been described previously, including $lpfA_{O26}$ and $lpfA_{O113}$, described by Toma et al. (41) and Doughty et al. (12), respectively.

* Corresponding author. Mailing address: Department of Infectious and Parasitic Diseases—Bacteriology, Veterinary Faculty, University of Liège, Liège B4000, Belgium. Phone: 32 4 366 40 52. Fax: 32 4 366 42 63. E-mail: mbardiau@ulg.ac.be.

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In addition, other putative adhesins have been described, as follows: a 67-kDa adherence-conferring protein (Iha) similar to *Vibrio cholerae* IrgA confers the capacity to adhere to epithelial cells in a diffuse pattern (38); Efa1 (EHEC factor for adherence), described by Nicholls et al. (27), mediates the binding of bacteria to CHO cells in vitro; ToxB, a protein encoded by a gene located on the 93-kb pO157 plasmid, is required for full adherence of the EHEC O157:H7 Sakai strain (39); Saa is an autoagglutinating adhesin identified in locus of enterocyte effacement-negative verotoxigenic *E. coli* strains (29); EibG is a protein responsible for the chain-like adherence phenotype of Saa-negative verotoxigenic *E. coli* strains (22); Paa (porcine attaching and effacing-associated) adhesin, described by An et al. (1), is involved in the early steps of the adherence mechanism of porcine EPEC strains (2); and the hemorrhagic coli pilus (HCP), whose inactivation of the main subunit (*hcpA* gene) reduces adherence to cultured human intestinal and bovine renal epithelial cells and to porcine and bovine gut explants, was observed in EHEC O157:H7 (46).

The aim of this study was to establish the prevalence in bovine and human EPEC and EHEC strains belonging to the O26 serogroup of a total of 23 putative adhesins previously described for EHEC strains and of four fimbrial and afimbrial adhesins associated with bovine necrotoxigenic *E. coli* (NTEC) (36). The presence of these genes was correlated, on the one hand, with the source of isolation, and on the other hand, with EHEC/EPEC virulence factors (*eae*, stx_1, stx_2, and EHEC *hlyA*).

MATERIALS AND METHODS

Bacterial strains. A total of 77 strains of serogroup O26 isolated in the United States, Ireland, Belgium, France, Japan, and Brazil were studied (Table 1) and included 5 non-EPEC/non-EHEC strains, 28 EPEC strains, and 44 EHEC strains. Forty-four strains were isolated from the feces or intestines of young calves (1 non-EPEC/non-EHEC, 18 EPEC, and 25 EHEC strains), and 33 strains were isolated from humans (4 non-EPEC/non-EHEC, 10 EPEC, and 19 EHEC strains). Most of the strains had been described previously (37), but their pathotype (EPEC or EHEC) and serotype O26:H11 status were confirmed by PCR for the stx_1, stx_2, *eae*, EHEC *hlyA*, $wzx\text{-}wzy_{O26}$, and $fliC_{H11}$ genes (Table 1) (10, 13, 15).

The following positive controls were used: strains EH017 and EH383 (for the 14 putative fimbrial gene clusters, *toxB* gene, *paa* gene, *iha* gene, and *hcpA* gene), strain 239KH89 (for the *afa8-E* gene), strain 25KH9 (for the *f17A* gene), and strain 31A (for the *clpG* and *clpE* genes). The nonpathogenic strain HS (O9:H4) was used as a negative control.

PCR. All primers used in this study are listed in Table 2. DNA templates were prepared by boiling, as previously described (10). For PCRs, the following mixture was used: 1 U of *Taq* DNA polymerase (New England Biolabs), 5 μl of a mixture with a 2 mM concentration of each deoxynucleoside triphosphate, 5 μl of 10× ThermoPol reaction buffer [20 mM Tris-HCl (pH 8.8, 25°C), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100], 5 μl of each primer (10 μM), and 3 μl of DNA template in a total volume of 50 ml. All PCR conditions have been described previously (Table 2), and the annealing temperatures are listed in Table 2. Some PCRs were performed in duplicate to confirm the results.

DNA sequencing. The DNA fragments were purified using a NucleoSpin Extract II kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. Sequencing of the two DNA strands was performed by the dideoxynucleotide triphosphate chain termination method with an ABI 3730 capillary sequencer and a BigDye Terminator kit, version 3.1 (Applied Biosystems), at the Groupe Interdisciplinaire de Génoprotéomique Appliquée (University of Liège, Belgium). Sequence analysis was performed using Vector NTI 10.1.1 (Invitrogen).

Statistical analysis. Fisher's exact test was performed to assess statistical differences ($P < 0.01$).

RESULTS

Distribution of putative EPEC and EHEC adhesin-encoding genes. The distribution of putative EPEC and EHEC adhesin-encoding genes is shown in Table 3. The following genes were detected in the majority of the strains: *loc3* (in 100% of the strains), *loc14* (in 100% of the strains), *loc5* (in 99% of the strains), *loc7* (in 99% of the strains), *loc8* (in 83% of the strains), *loc11* (in 97% of the strains), *efa1* (in 92% of the strains), *toxB* (in 79% of the strains), *paa* (in 97% of the strains), *iha* (in 92% of the strains), $lpfA_{O26}$ (in 94% of the strains), and $lpfA_{O113}$ (in 95% of the strains).

In addition, a few strains were positive for some adhesins that have not yet been described for EPEC/EHEC O26 strains, including *hcpA* (four strains), *loc4* (two strains), *loc9* (three strains), and *loc10* (two strains). These amplicons were sequenced for further identification and comparison. The four *hcpA* amplicon sequences had 100% identity with the *hcpA* gene. The two *loc4* amplicon sequences were 69% identical to the *loc4* gene of the positive control and 100% identical to the *ybgD* gene of *Shigella*, coding for a putative fimbrial subunit-like protein. Nevertheless, the two usher protein-encoding genes (*loc4 usher1* and *loc4 usher2* genes) associated with the *loc4* fimbriae were not detected in those two strains. The three *loc9* amplicon sequences were identical to the amplicon sequence of the positive control. The two usher protein-encoding genes and the fimbrial subunit-encoding gene (*loc9 usher1*, *loc9 usher2*, and *loc9* fimbrial subunit genes) were also detected in the three strains by PCR. One of the two *loc10* amplicon sequences was identical to the *loc10* gene, whereas the second one had only 74% identity. The latter sequence was 99% identical to the *yfcV* gene, coding for a putative fimbrial subunit of *E. coli* UTI89. However, the two usher protein-encoding genes (*loc4 usher1* and *loc4 usher2* genes) associated with the *loc10* fimbriae were detected in neither strain. Three genes, *loc1*, *loc2*, and *loc6*, were not detected in any of the strains.

Distribution of putative NTEC adhesin-encoding genes. Fimbrial and afimbrial adhesins (Afa8-E, F17A, ClpG, and ClpE) associated with bovine NTEC strains had previously been used as search targets for 24 EHEC and EPEC strains of serogroup O26 (36). We extended the adhesin search to 77 strains. Four strains were *afa8-E* positive, 2 strains were *f17A* positive, 1 strain was *clpG* positive, and 18 strains (25%) were *clpE* positive. These amplicons were also further identified and compared after being sequenced. The *afa8-E* and *f17A* amplicon sequences were identical to the amplicon sequences of the respective positive controls. One *clpE* amplicon sequence was 100% identical to the *clpE* gene. The strain carrying this sequence was also positive for the *clpG* gene. The other 17 *clpE* amplicon sequences had 100% identity with each other, 90% identity with the *clpE* gene, and 100% identity with the *ldaE* gene, coding for the chaperone of the "locus for diffuse adherence," described by Scaletsky et al. (33). Eleven of the 17 strains carrying these sequences were also positive for the *ldaG* gene, coding for the main subunit of the locus for diffuse adherence.

Distribution of putative adhesins according to source of isolation (cattle or humans), geographical origin, and pathotype. Several adhesin-encoding genes (*loc8*, *loc11*, *efa1*, *toxB*, *iha*, $lpfA_{O26}$, $lpfA_{O113}$, and *ldaE*) were statistically associated

TABLE 1. Serotypes, sources of isolation, geographical origins, and pathotypes of tested strains

Strain	Host	Status of host[a]	Origin	EPEC or EHEC	Presence of adhesin gene or variant (for *stx*)				
					eae	*stx*	EHEC *hlyA*	*wzx-wzy*$_{O26}$	*fliC*$_{H11}$
TC3108	Cattle	Healthy	United States	EHEC	+	1	−	+	+
TC3109	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3117	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3180	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3269	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3273	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3302	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3305	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3375	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3380	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3629	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3630	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3631	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3632	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3656	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC3657	Cattle	Healthy	United States	EHEC	+	1	+	+	+
TC6169	Cattle	Diarrheic	United States	EHEC	+	1	+	+	+
4276	Cattle	Diarrheic	Ireland	EHEC	+	1	+	+	+
A39	Cattle	?	?	EHEC	+	1	+	+	+
357S89	Cattle	Diarrheic	Belgium	EHEC	+	1	+	+	+
379S89	Cattle	Diarrheic	Belgium	EHEC	+	1	+	+	+
122	Cattle	?	?	EHEC	+	1	+	+	+
63	Cattle	?	?	EHEC	+	1	+	+	+
A14	Cattle	?	?	EHEC	+	1	+	+	+
331S89	Cattle	Diarrheic	Belgium	EHEC	+	1	+	+	+
EH031	Human	Diarrheic	Belgium	EHEC	+	1	+	+	+
EH182	Human	Diarrheic	Belgium	EHEC	+	1	+	+	+
EH193	Human	Diarrheic	Belgium	EHEC	+	2	+	+	+
EH296	Human	Diarrheic	Belgium	EHEC	+	2	+	+	+
EH298	Human	Diarrheic	Belgium	EHEC	+	2	+	+	+
EH196	Human	Diarrheic	Belgium	EHEC	+	2	+	+	+
EH284	Human	Diarrheic	Belgium	EHEC	+	1	+	+	+
EH322	Human	Diarrheic	Belgium	EHEC	+	1	+	+	+
EH324	Human	Diarrheic	Belgium	EHEC	+	1	+	+	+
TC5710	Human	HUS	United States	EHEC	+	1 and 2	−	+	+
TC5711	Human	HUS	United States	EHEC	+	1	+	+	+
TC6168	Human	Diarrheic	United States	EHEC	+	1	−	+	+
02/113	Human	?	France	EHEC	+	1	+	+	+
99/109	Human	?	France	EHEC	+	2	+	+	+
03/151	Human	?	France	EHEC	+	1	+	+	+
03/139	Human	?	France	EHEC	+	1	+	+	+
99/145	Human	?	France	EHEC	+	1	+	+	+
99/147	Human	?	France	EHEC	+	1	+	+	+
11368	Human	Diarrheic	Japan	EHEC	+	1	+	+	+
TC1988	Human	?	Brazil	EPEC	+	−	+	+	+
TC3145	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC3486	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC3748	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC4004	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC4219	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC4221	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC3848	Cattle	Healthy	United States	EPEC	+	−	+	+	+
TC659	Cattle	Diarrheic	United States	EPEC	+	−	+	+	+
333KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	+	+	+
334KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
351KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
352KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	+	+	+
631KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
C15333	Cattle	?	?	EPEC	+	−	−	+	+
331KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
335KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
355KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
354KH91	Cattle	Diarrheic	Belgium	EPEC	+	−	−	+	+
TC6165	Human	Diarrheic	United States	EPEC	+	−	+	+	+
TC6166	Human	Diarrheic	United States	EPEC	+	−	+	+	+
TC6167	Human	Healthy	United States	EPEC	+	−	−	+	+
03/178	Human	?	France	EPEC	+	−	+	+	+
00/054	Human	?	France	EPEC	+	−	+	+	+
00/106	Human	?	France	EPEC	+	−	−	+	+
00/113	Human	?	France	EPEC	+	−	−	+	+
02/145	Human	?	France	EPEC	+	−	−	+	+
02/057	Human	?	France	EPEC	+	−	−	+	+
T282	Cattle	Diarrheic	United States	Non-EPEC/non-EHEC	−	−	−	+	+
00/103	Human	?	France	Non-EPEC/non-EHEC	−	−	−	+	+
00/130	Human	?	France	Non-EPEC/non-EHEC	−	−	−	+	+
03/023	Human	?	France	Non-EPEC/non-EHEC	−	−	−	+	+
C4071	Human	?	France	Non-EPEC/non-EHEC	−	−	−	+	+

[a] HUS, hemolytic-uremic syndrome.

TABLE 2. Primers used in this study

Primer	Sequence (5′ to 3′)	Target	Annealing temp (°C)	Amplicon size (bp)	Reference
loc1-F	CGACAACGTTGATGTTTAGC	*loc1* main subunit	48	300–500	21
loc1-R	GCCTTTTGTAACAGGATTGC				
loc2-F	GGTATGCATAGCGTTACC	*loc2* main subunit	42	300–500	21
loc2-R	CTGCTGGCAAATCTTATGC				
loc3-F	GCGGTACAATTCACTTTGAAGG	*loc3* main subunit	53	300–500	21
loc3-R	CATTTGCTTGCCCTGCTGATGC				
loc4-F	GCCATATCTCTACTATTCGC	*loc4* main subunit	43	300–500	21
loc4-R	GTTATCCATCTGTTCCATCC				
loc5-F	CTGTGGTATGTGCAACGTCC	*loc5* main subunit	51	300–500	21
loc5-R	CCCCGTAGCGATATAATCAAC				
loc6-F	CCTACAGTCACTTTTCAGGG	*loc6* main subunit	44	300–500	21
loc6-R	GATTAATTAGAGGTAGCTCAGG				
loc7-F	CTTCATTTAATCAGGCAGCC	*loc7* main subunit	47	300–500	21
loc7-R	GAGTACCATACTGTGTAATATTTGC				
loc8-F	GGTGATGAATCAGTAACGACC	*loc8* main subunit	48	300–500	21
loc8-R	GTGCCATCAATCAAGTCGG				
loc9-F	CACCATGTACATTTGTCGC	*loc9* main subunit	46	300–500	21
loc9-R	CAGTACGTCACTGCTATCTCC				
loc10-F	GCTGCAACAATGGTAATGGG	*loc10* main subunit	53	300–500	21
loc10-R	GTAATCTGGAAGGTCGTGTTGGC				
loc11-F	CTTTTCGCAGGTAATGCCG	*loc11* main subunit	50	300–500	21
loc11-R	GATTTCGGATGCTTCAACG				
loc12-F	GTGGTATCGCAATCTTCC	*loc12* main subunit	42	300–500	21
loc12-R	GGTAAAGTAGAGAACCG				
loc13-F	GATTGTAGGAGCATTAGCG	*loc13* main subunit	45	300–500	21
loc13-R	CTATCGATCTGACTCAATGCC				
loc14-F	GTCGTTGCTGCCAATGTTTGC	*loc14* main subunit	48	300–500	21
loc14-R	GAAATGTAGCGAAGTAGAGCC				
LpfA-O26-F	GTT CTG TTT GCC TTA TCT GC	$lpfA_{O26}$	52	509	40, 41
LpfA-O26-R	TAA GTC AGG TTG AAG TCG AC				
LpfA-O113-F	ATGAAGCGTTAATATTATAG	$lpfA_{O113}$	50	573	28
LpfA-O113-R	TTATTTCTTATATTCGAC				
efa1-F	TAA GCG AGC CCT GAT AAG CA	*efa1*	55	630	17
efa1-R	CGT GTT GCT TGC CTT TGC				
toxB-F	ATACCTACCTGCTCTGGATTGA	*toxB*	55	602	41
toxB-R	TTCTTACCTGATCTGATGCAGC				
afa8-E-F	CTAACTTGCCATGCTGTGACAGTA	*afa8-E*	65	302	36
afa8-E-R	TTATCCCCTGCGTAGTTGTGAATC				
f17A-F	GCAGAAAATTCAATTTATCCTTGG	*f17A*	55	537	36
f17A-R	CTGATAAGCGATGGTGTAATTAAC				
clpE-F	GGTCAGGCCTGGGTGGACAATATC	*clpE*	58	240	36
clpE-R	GCGATAGAACAGTTTCAGCTTCGT				
clpG-F	GGGCGCTCTCTCCTTCAAC	*clpG*	55	403	36
clpG-R	CGCCCTAATTGCTGGCGAC				
paa-F	TCAGAACAATCTGCTCTGGCTA	*paa*	52	413	19
paa-R	CACGTAGTCTGGCGCTATTTC				
iha-F	CAAATGGCTCTCTTCCGTCAATGC	*iha*	59	925	36
iha-F	CAGGTCGGGGTTACCAAGT				
saa-F	CGTGATGAACAGGCTATTGC	*saa*	50	119	18
saa-R	ATGGACATGCCTGTGGCAAC				
eibG-F	ATCGGCTTTCATCGCATCAGGAC	*eibG*	60	?	22
eibG-R	CCACAAGGCGGGTATTCGTATC				
B52	AGGCTTCGTCACAGTTG	*eaeA*	50	570	10
B53	CCATCGTCACCAGAGGA				
B54	AGAGCGATGTTACGGTTTG	*sltI*	50	388	10
B55	TTGCCCCCAGAGTGGATG				
B56	TGGGTTTTTCTTCGGTATC	*sltII*	50	807	10
B57	GACATTCTGGTTGACTCTCTT				
EHEC-hlyA-F	ACGATGTGGTTTATTCTGGA	EHEC *hlyA*	58	165	15
EHEC-hlyA-R	CTTCACGTGACCATACATAT				
wzx-wzyO26-F	AAATTAGAAGCGCGTTCATC	wzx-wzy_{O26}	56	596	13
wzx-wzyO26-R	CCCAGCAAGCCAATTATGACT				
fliC-H11-F	ACTGTTAACGTAGATAGC	$fliC_{H11}$	56	224	13
fliC-H11-R	TCAATTTCTGCAGAATATAC				
G98-F	TCGCTAGTTGCTGACAGATTT	*hcpA*	49	?	46
G99-R	AATGTCTGTTGTGTGCGACTG				
ldaG-F	ATGAAAAAGACACTATTAGCACTGG	*ldaG*	48	?	33
ldaG-R	TGAATCTCCCAGCCAAAA				
loc4U1-F	CCTCTATGTCGCACCACAC	*loc4 usher1*	48	300–500	21
loc4U1-R	GTCGGCGTCATTGTATTTG				
loc4U2-F	GCTCAATCAAAAAGGCGTC	*loc4 usher2*	46	300–500	21
loc4U2-R	CCGTTATTCCACGTTGAG				
loc9U1-F	GGATAATAATCCTGGTGAGTG	*loc9 usher1*	46	300–500	21
loc9U1-R	CACTTTGCTTGCTCGCAC				
loc9U2-F	GGAACCGCGATCAGTTTA	*loc9 usher2*	46	300–500	21
loc9U2-R	GCTGTCCATCGGATCTTA				
loc9fim-F	GCATTGAAGTTTAACTGGTG	*loc9* fimbrial subunit	44	300–500	21
loc9fim-R	CCTGGTTTATCTGTTTTCC				
loc10U1-F	GATGACGATGTTATCAACGG	*loc10 usher1*	48	300–500	21
loc10U1-R	GAAGAAACCGCCTTCCAC				
loc10U2-F	GGAGTTATGTCAATGCCT	*loc10 usher2*	42	300–500	21
loc10U2-R	GGATCCCAGTTGATGTCG				

TABLE 3. Distribution of putative adhesin genes

Strain type	No. of strains tested	No. (%) of strains positive for gene by PCR												
		loc1	*loc2*	*loc3*	*loc4*	*loc5*	*loc6*	*loc7*	*loc8*	*loc9*	*loc10*	*loc11*	*loc12*	*loc13*
Total	77	0 (0)	0 (0)	77 (100)	2 (3)	76 (99)	0 (0)	76 (99)	64 (83)	3 (4)	2 (3)	75 (97)	0 (0)	0 (0)
Bovine strains	44	0 (0)	0 (0)	44 (100)	1 (2)	43 (98)	0 (0)	43 (98)	38 (86)	2 (5)	1 (2)	44 (100)	0 (0)	0 (0)
Human strains	33	0 (0)	0 (0)	33 (100)	1 (3)	33 (100)	0 (0)	33 (100)	26 (79)	1 (3)	1 (3)	31 (94)	0 (0)	0 (0)
stx_1-positive strains	38	0 (0)	0 (0)	38 (100)	1 (3)	38 (100)	0 (0)	37 (97)	30 (79)	2 (5)	0 (0)	38 (100)	0 (0)	0 (0)
stx_2-positive strains	5	0 (0)	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)	5 (100)	4 (80)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)
stx_1- and stx_2-positive strains	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
EPEC strains	28	0 (0)	0 (0)	28 (100)	0 (0)	27 (96)	0 (0)	28 (100)	27 (96)	0 (0)	1 (4)	28 (100)	0 (0)	0 (0)
Non-EPEC/non-EHEC strains	5	0 (0)	0 (0)	5 (100)	1 (20)	5 (100)	0 (0)	5 (100)	2 (40)	1 (20)	0 (0)	3 (600)	0 (0)	0 (0)
EHEC strains	44	0 (0)	0 (0)	44 (100)	1 (2)	44 (100)	0 (0)	43 (98)	35 (80)	2 (5)	1 (2)	44 (100)	0 (0)	0 (0)
Belgian strains	21	0 (0)	0 (0)	21 (100)	0 (0)	20 (95)	0 (0)	21 (100)	17 (81)	0 (0)	1 (5)	21 (100)	0 (0)	0 (0)
Brazilian strains	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
French strains	16	0 (0)	0 (0)	16 (100)	1 (6)	16 (100)	0 (0)	16 (100)	12 (75)	1 (6)	0 (0)	14 (88)	0 (0)	0 (0)
Irish strains	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Japanese strains	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
American strains	32	0 (0)	0 (0)	32 (100)	0 (0)	32 (100)	0 (0)	32 (100)	27 (84)	1 (3)	1 (3)	32 (100)	0 (0)	0 (0)
Strains of unknown origin	5	0 (0)	0 (0)	5 (100)	1 (20)	5 (100)	0 (0)	4 (80)	5 (100)	1 (20)	0 (0)	5 (100)	0 (0)	0 (0)
European strains	38	0 (0)	0 (0)	38 (100)	1 (3)	37 (97)	0 (0)	38 (100)	30 (79)	1 (3)	1 (3)	36 (95)	0 (0)	0 (0)
Diarrheic bovine strains	16	0 (0)	0 (0)	16 (100)	0 (0)	15 (94)	0 (0)	16 (100)	15 (94)	1 (6)	1 (6)	16 (100)	1 (6)	0 (0)
Healthy bovine strains	23	0 (0)	0 (0)	23 (100)	0 (0)	23 (100)	0 (0)	23 (100)	18 (78)	0 (0)	0 (0)	23 (100)	0 (0)	0 (0)

with the EHEC/EPEC strains in comparison with the non-EPEC/non-EHEC strains ($P < 0.01$), and the $lpfA_{O26}$ gene was marginally statistically associated with bovine EHEC/EPEC strains in comparison with human strains ($P = 0.012$). Moreover, the *ldaE* gene was statistically associated with the bovine EHEC/EPEC strains isolated from diarrheic calves in comparison with the other bovine strains ($P < 0.01$) but not in comparison with the human EHEC/EPEC strains. The *f17A*, *afa8-E*, and *clpG*/*clpE* genes were detected only in the EPEC strains isolated from diarrheic calves in Belgium, though this association was not statistically significant due to the small number of positive strains. For the other adhesins, no relationship was observed between the source (cattle or humans), the geographical origin, or the pathotype of the strains and their prevalence.

DISCUSSION

Studies on the prevalence of putative EHEC adhesins have focused mostly on O157:H7 strains and more rarely on a few non-O157 strains. However, non-O157:H7 serogroups (such as O26, O145, O111, and O103) are frequently associated with severe illness in humans (5), and in many countries, O26 strains are the second most prevalent serogroup of EHEC strains (4). Moreover, O26 strains possess the particularity of producing disease in both humans and calves (23). Evidence also exists that human and bovine EHEC O26 strains are heterogeneous, leading to the hypothesis that at least some of them may be host specific. The step involving the initial adherence of bacteria to intestinal cells could be the basis of such host specificity, as is the case with other pathogenic *E. coli* strains and virulence factors, such as F18a of porcine verotoxigenic *E. coli*, AF/R1 and AF/R2 of rabbit enteropathogenic *E. coli*, F4 and F6 of porcine enterotoxigenic *E. coli*, etc. (16, 25). Therefore, 77 EHEC and EPEC O26 strains recovered from different sources (human or bovine) in different countries were tested by PCR for the presence of genes coding for 27 putative adhesins previously described or used as search targets for EHEC and EPEC strains (these previous attempts had either not involved O26 strains or used only a limited number of those strains). This is the first time that the distribution of so many putative adhesin-encoding genes has been described for such a large collection of EPEC/EHEC O26 strains.

According to the PCR results, the following four "groups" of adhesin genes exist: adhesin genes present in all O26 strains (*loc3* and *loc14*), adhesin genes present in most O26 strains (*loc5*, *loc7*, *loc8*, *loc11*, *efa1*, *toxB*, *paa*, *iha*, $lpfA_{O26}$, and $lpfA_{O113}$), adhesin genes present in a few O26 strains (*loc4*, *loc9*, *loc10*, *afa8-E*, *f17A*, *ldaE*, *clpE*/*clpG*, and *hcpA*), and adhesin genes not present in O26 strains (*loc1*, *loc2*, *loc6*, *loc12*, *loc13*, *saa*, and *eibG*). The common adhesin profile of EHEC/EPEC O26 strains is therefore characterized by the presence of *loc3*, *loc5*, *loc7*, *loc11*, *loc14*, *paa*, *efa1*, *iha*, $lpfA_{O26}$, and $lpfA_{O113}$ genes and the absence of *loc1*, *loc2*, *loc6*, *loc12*, *loc13*, *saa*, and *eibG* genes. Interestingly, the *loc8*, *loc11*, *afa8-E*, *f17A*, *ldaE*, *efa1*, *toxB*, *iha*, $lpfA_{O26}$, and $lpfA_{O113}$ genes were more frequent in EHEC/EPEC strains than in other strains. Also, several strains were found to be positive for some adhesin genes that have so far not been described for EPEC/EHEC O26 strains, such as *hcpA*, *loc4*, *loc9*, *loc10*, *afa8-E*, *f17A*, and *clpE*/*clpG*.

Nevertheless, none of the adhesins studied was significantly associated with bovine or human strains ($P > 0.01$). On the other hand, the *ldaE* gene was found to be statistically associated with EHEC/EPEC O26 strains isolated from diarrheic calves in comparison with strains isolated from healthy calves. These *ldaE*-positive strains may therefore represent a subgroup possessing the specific property of producing diarrhea in young calves (without presuming their capacity to cause disease in humans).

Since not all EHEC/EPEC strains isolated from diarrheic calves are positive for *ldaE*, the capacity of the other strains to cause diarrhea in young calves must be based upon another property, such as (i) other, rarer adhesins (*afa8-E*, *f17A*, *clpG*/*clpE*, etc.); (ii) the existence of differences in the sequences of genes coding for some adhesins present in human and bovine strains, resulting in host and tissue tropism, as already described for other families of fimbrial (P family) and afimbrial (AFA family) adhesins (3, 16), which can be detected only after sequencing of the whole encoding genes; (iii) variation in

TABLE 3—*Continued*

No. (%) of strains positive for gene by PCR													
loc14	*efa1*	*toxB*	*afa8-E*	*f17A*	*ldaE*	*clpE*/*clpG*	*paa*	*iha*	*saa*	*eibG*	$lpfA_{O26}$	$lpfA_{O113}$	*hcpA*
77 (100)	71 (92)	61 (79)	4 (5)	2 (3)	17 (22)	1 (1)	75 (97)	71 (92)	0 (0)	0 (0)	72 (94)	73 (95)	4 (5)
44 (100)	43 (98)	37 (84)	4 (9)	2 (5)	11 (25)	1 (2)	43 (98)	43 (98)	0 (0)	0 (0)	44 (100)	43 (98)	3 (7)
33 (100)	28 (85)	24 (73)	0 (0)	0 (0)	6 (18)	0 (0)	32 (97)	28 (85)	0 (0)	0 (0)	28 (85)	30 (91)	1 (3)
38 (100)	38 (100)	36 (95)	0 (0)	0 (0)	3 (8)	0 (0)	38 (100)	38 (100)	0 (0)	0 (0)	38 (100)	38 (100)	1 (3)
5 (100)	5 (100)	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)	5 (100)	0 (0)	0 (0)	5 (100)	5 (100)	0 (0)
1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
28 (100)	27 (96)	19 (68)	4 (14)	2 (7)	14 (50)	1 (4)	27 (96)	26 (93)	0 (0)	0 (0)	27 (96)	27 (96)	2 (7)
5 (100)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	4 (80)	1 (20)	0 (0)	0 (0)	1 (20)	2 (40)	1 (20)
44 (100)	44 (100)	41 (93)	0 (0)	0 (0)	3 (7)	0 (0)	44 (100)	44 (100)	0 (0)	0 (0)	44 (100)	44 (100)	1 (2)
21 (100)	21 (100)	16 (76)	4 (19)	2 (10)	7 (33)	1 (5)	20 (95)	20 (95)	0 (0)	0 (0)	21 (100)	20 (95)	3 (14)
1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
16 (100)	11 (69)	9 (56)	0 (0)	0 (0)	3 (19)	0 (0)	15 (94)	11 (69)	0 (0)	0 (0)	11 (69)	13 (81)	1 (6)
1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
32 (100)	31 (97)	29 (91)	0 (0)	0 (0)	3 (9)	0 (0)	32 (100)	32 (100)	0 (0)	0 (0)	32 (100)	32 (100)	0 (0)
5 (100)	5 (100)	4 (80)	0 (0)	0 (0)	4 (80)	0 (0)	5 (100)	5 (100)	0 (0)	0 (0)	5 (100)	5 (100)	0 (0)
38 (100)	33 (87)	26 (68)	4 (11)	2 (5)	10 (26)	1 (3)	36 (95)	32 (84)	0 (0)	0 (0)	33 (87)	34 (89)	0 (0)
16 (100)	15 (94)	11 (69)	4 (25)	2 (13)	7 (44)	1 (6)	15 (94)	15 (94)	12 (75)	0 (0)	16 (100)	15 (94)	3 (19)
23 (100)	23 (100)	22 (96)	0 (0)	0 (0)	0 (0)	0 (0)	23 (100)	23 (100)	19 (83)	0 (0)	23 (100)	23 (100)	0 (0)

the expression of some adhesin-encoding genes according to the growth environment (bovine or human intestine, intestinal segment, age of the host, etc.), as observed for other genes (11); or (iv) properties other than adherence, such as intermediate metabolism, which allows bacteria to be better adapted to a bovine intestinal environment, such as the young calf intestine (14, 44).

In conclusion, the answer to the question of host specificity of bovine and human EHEC/EPEC O26 strains may simply be that several subgroups of strains exist depending on the presence or absence of one or several properties allowing the pathogens to colonize (or hampering them from doing so) one specific host intestine (young calf, adult cattle, and/or human intestine) and allowing them to cause diarrhea. Only adherence experiments with enterocytes from humans and bovines and/or in vivo challenge of young calves with wild-type strains and mutants would bring final answers to these questions.

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REFERENCES

1. **An, H., J. Fairbrother, C. Desautels, and J. Harel.** 1999. Distribution of a novel locus called Paa (porcine attaching and effacing associated) among enteric Escherichia coli. Adv. Exp. Med. Biol. **473:**179–184.
2. **Batisson, I., M. P. Guimond, F. Girard, H. An, C. Zhu, E. Oswald, J. M. Fairbrother, M. Jacques, and J. Harel.** 2003. Characterization of the novel factor *paa* involved in the early steps of the adhesion mechanism of attaching and effacing *Escherichia coli*. Infect. Immun. **71:**4516–4525.
3. **Berendson, R., C. P. Cheney, P. A. Schad, and E. C. Boedeker.** 1983. Species-specific binding of purified pili (AF/R1) from the Escherichia coli RDEC-1 to rabbit intestinal mucosa. Gastroenterology **85:**837–845.
4. **Bielaszewska, M., W. Zhang, A. Mellmann, and H. Karch.** 2007. Enterohaemorrhagic Escherichia coli O26:H11/H−: a human pathogen in emergence. Berl. Munch. Tierarztl. Wochenschr. **120:**279–287.
5. **Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine.** 2005. Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983–2002. J. Infect. Dis. **192:**1422–1429.
6. **Campos, L. C., M. R. Franzolin, and L. R. Trabulsi.** 2004. Diarrheagenic Escherichia coli categories among the traditional enteropathogenic E. coli O serogroups—a review. Mem. Inst. Oswaldo Cruz **99:**545–552.
7. **Caprioli, A., S. Morabito, H. Brugëre, and E. Oswald.** 2005. Enterohaemorrhagic Escherichia coli: emerging issues on virulence and modes of transmission. Vet. Res. **36:**289–311.
8. **Chen, H., and G. Frankel.** 2005. Enteropathogenic Escherichia coli: unravelling pathogenesis. FEMS Microbiol. Rev. **29:**83–98.
9. **China, B., V. Pirson, and J. Mainil.** 1998. Prevalence and molecular typing of attaching and effacing Escherichia coli among calf populations in Belgium. Vet. Microbiol. **63:**249–259.
10. **China, B., V. Pirson, and J. Mainil.** 1996. Typing of bovine attaching and effacing *Escherichia coli* by multiplex in vitro amplification of virulence-associated genes. Appl. Environ. Microbiol. **62:**3462–3465.
11. **de Sablet, T., C. Chassard, A. Bernalier-Donadille, M. Vareille, A. P. Gobert, and C. Martin.** 2009. Human microbiota-secreted factors inhibit Shiga toxin synthesis by enterohemorrhagic *Escherichia coli* O157:H7. Infect. Immun. **77:**783–790.
12. **Doughty, S., J. Sloan, V. Bennett-Wood, M. Robertson, R. M. Robins-Browne, and E. L. Hartland.** 2002. Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. Infect. Immun. **70:**6761–6769.
13. **Durso, L. M., J. L. Bono, and J. E. Keen.** 2005. Molecular serotyping of *Escherichia coli* O26:H11. Appl. Environ. Microbiol. **71:**4941–4944.
14. **Dziva, F., P. M. van Diemen, M. P. Stevens, A. J. Smith, and T. S. Wallis.** 2004. Identification of Escherichia coli O157:H7 genes influencing colonization of the bovine gastrointestinal tract using signature-tagged mutagenesis. Microbiology **150:**3631–3645.
15. **Fagan, P. K., M. A. Hornitzky, K. A. Bettelheim, and S. P. Djordjevic.** 1999. Detection of Shiga-like toxin (stx_1 and stx_2), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl. Environ. Microbiol. **65:**868–872.
16. **Fairbrother, J. M., E. Nadeau, and C. L. Gyles.** 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. **6:**17–39.
17. **Janka, A., M. Bielaszewska, U. Dobrindt, and H. Karch.** 2002. Identification and distribution of the enterohemorrhagic Escherichia coli factor for adherence (efa1) gene in sorbitol-fermenting Escherichia coli O157:H. Int. J. Med. Microbiol. **292:**207–214.
18. **Jenkins, C., N. T. Perry, T. Cheasty, D. J. Shaw, G. Frankel, G. Dougan, G. J. Gunn, H. R. Smith, A. W. Paton, and J. C. Paton.** 2003. Distribution of the *saa* gene in strains of Shiga toxin-producing *Escherichia coli* of human and bovine origins. J. Clin. Microbiol. **41:**1775–1778.
19. **Leclerc, S., P. Boerlin, C. Gyles, J. D. Dubreuil, M. Mourez, J. M. Fairbrother, and J. Harel.** 2007. paa, originally identified in attaching and effacing Escherichia coli, is also associated with enterotoxigenic E. coli. Res. Microbiol. **158:**97–104.
20. **Low, A., F. Dziva, A. Torres, J. Martinez, T. Rosser, S. Naylor, K. Spears, N. Holden, A. Mahajan, J. Findlay, J. Sales, D. Smith, J. Low, M. Stevens, and D. Gally.** 2006. Cloning, expression, and characterization of fimbrial operon F9 from enterohemorrhagic *Escherichia coli* O157:H7. Infect. Immun. **74:**2233–2244.
21. **Low, A., N. Holden, T. Rosser, A. Roe, C. Constantinidou, J. Hobman, D. Smith, J. Low, and D. Gally.** 2006. Analysis of fimbrial gene clusters and

their expression in enterohaemorrhagic Escherichia coli O157:H7. Environ. Microbiol. **8:**1033–1047.
22. **Lu, Y., S. Iyoda, H. Satou, K. Itoh, T. Saitoh, and H. Watanabe.** 2006. A new immunoglobulin-binding protein, EibG, is responsible for the chain-like adhesion phenotype of locus of enterocyte effacement-negative, Shiga toxin-producing *Escherichia coli*. Infect. Immun. **74:**5747–5755.
23. **Mainil, J.** 1999. Shiga/verocytotoxins and Shiga/verotoxigenic Escherichia coli in animals. Vet. Res. **30:**235–257.
24. **Mainil, J. G., and G. Daube.** 2005. Verotoxigenic Escherichia coli from animals, humans and foods: who's who? J. Appl. Microbiol. **98:**1332–1344.
25. **Nagy, B., and P. Z. Fekete.** 1999. Enterotoxigenic Escherichia coli (ETEC) in farm animals. Vet. Res. **30:**259–284.
26. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
27. **Nicholls, L., T. H. Grant, and R. M. Robins-Browne.** 2000. Identification of a novel genetic locus that is required for in vitro adhesion of a clinical isolate of enterohaemorrhagic Escherichia coli to epithelial cells. Mol. Microbiol. **35:**275–288.
28. **Osek, J., M. Weiner, and E. L. Hartland.** 2003. Prevalence of the lpfO113 gene cluster among Escherichia coli O157 isolates from different sources. Vet. Microbiol. **96:**259–266.
29. **Paton, A. W., P. Srimanote, M. C. Woodrow, and J. C. Paton.** 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infect. Immun. **69:**6999–7009.
30. **Pawar, D. M., M. L. Rossman, and J. Chen.** 2005. Role of curli fimbriae in mediating the cells of enterohaemorrhagic Escherichia coli to attach to abiotic surfaces. J. Appl. Microbiol. **99:**418–425.
31. **Reid, S. D., C. J. Herbelin, A. C. Bumbaugh, R. K. Selander, and T. S. Whittam.** 2000. Parallel evolution of virulence in pathogenic Escherichia coli. Nature **406:**64–67.
32. **Roe, A. J., C. Currie, D. G. Smith, and D. L. Gally.** 2001. Analysis of type 1 fimbriae expression in verotoxigenic Escherichia coli: a comparison between serotypes O157 and O26. Microbiology **147:**145–152.
33. **Scaletsky, I. C., J. Michalski, A. G. Torres, M. V. Dulguer, and J. B. Kaper.** 2005. Identification and characterization of the locus for diffuse adherence, which encodes a novel afimbrial adhesin found in atypical enteropathogenic *Escherichia coli*. Infect. Immun. **73:**4753–4765.
34. **Shaikh, N., N. J. Holt, J. R. Johnson, and P. I. Tarr.** 2007. Fim operon variation in the emergence of enterohemorrhagic Escherichia coli: an evolutionary and functional analysis. FEMS Microbiol. Lett. **273:**58–63.
35. **Sonoda, C., A. Tagami, D. Nagatomo, S. Yamada, R. Fuchiwaki, M. Haruyama, Y. Nakamura, K. Kawano, M. Okada, Y. Shioyama, K. Iryoda, H. Wakamatu, and Y. Hidaka.** 2008. An enterohemorrhagic Escherichia coli O26 outbreak at a nursery school in Miyazaki, Japan. Jpn. J. Infect. Dis. **61:**92–93.
36. **Szalo, I. M., F. Goffaux, V. Pirson, D. Pièrard, H. Ball, and J. Mainil.** 2002. Presence in bovine enteropathogenic (EPEC) and enterohaemorrhagic (EHEC) Escherichia coli of genes encoding for putative adhesins of human EHEC strains. Res. Microbiol. **153:**653–658.
37. **Szalo, I. M., B. Taminiau, F. Goffaux, V. Pirson, J. McCappin, H. J. Ball, and J. G. Mainil.** 2004. 2F3 monoclonal antibody recognizes the O26 O-antigen moiety of the lipopolysaccharide of enterohemorrhagic *Escherichia coli* strain 4276. Clin. Diagn. Lab. Immunol. **11:**532–537.
38. **Tarr, P. I., S. S. Bilge, J. C. Vary, Jr., S. Jelacic, R. L. Habeeb, T. R. Ward, M. R. Baylor, and T. E. Besser.** 2000. Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect. Immun. **68:**1400–1407.
39. **Tatsuno, I., M. Horie, H. Abe, T. Miki, K. Makino, H. Shinagawa, H. Taguchi, S. Kamiya, T. Hayashi, and C. Sasakawa.** 2001. *toxB* gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. Infect. Immun. **69:**6660–6669.
40. **Toma, C., N. Higa, S. Iyoda, M. Rivas, and M. Iwanaga.** 2006. The long polar fimbriae genes identified in Shiga toxin-producing Escherichia coli are present in other diarrheagenic E. coli and in the standard E. coli collection of reference (ECOR) strains. Res. Microbiol. **157:**153–161.
41. **Toma, C., E. Martìnez Espinosa, T. Song, E. Miliwebsky, I. Chinen, S. Iyoda, M. Iwanaga, and M. Rivas.** 2004. Distribution of putative adhesins in different seropathotypes of Shiga toxin-producing *Escherichia coli*. J. Clin. Microbiol. **42:**4937–4946.
42. **Torres, A. G., J. A. Giron, N. T. Perna, V. Burland, F. R. Blattner, F. Avelino-Flores, and J. B. Kaper.** 2002. Identification and characterization of *lpfABCC′DE*, a fimbrial operon of enterohemorrhagic *Escherichia coli* O157:H7. Infect. Immun. **70:**5416–5427.
43. **Torres, A. G., K. J. Kanack, C. B. Tutt, V. Popov, and J. B. Kaper.** 2004. Characterization of the second long polar (LP) fimbriae of Escherichia coli O157:H7 and distribution of LP fimbriae in other pathogenic E. coli strains. FEMS Microbiol. Lett. **238:**333–344.
44. **van Diemen, P. M., F. Dziva, M. P. Stevens, and T. S. Wallis.** 2005. Identification of enterohemorrhagic *Escherichia coli* O26:H− genes required for intestinal colonization in calves. Infect. Immun. **73:**1735–1743.
45. **Wick, L. M., W. Qi, D. W. Lacher, and T. S. Whittam.** 2005. Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. J. Bacteriol. **187:**1783–1791.
46. **Xicohtencatl-Cortes, J., V. Monteiro-Neto, M. A. Ledesma, D. M. Jordan, O. Francetic, J. B. Kaper, J. L. Puente, and J. A. Girûn.** 2007. Intestinal adherence associated with type IV pili of enterohemorrhagic Escherichia coli O157:H7. J. Clin. Investig. **117:**3519–3529.