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Typing of Intimin (*eae*) Genes in Attaching and Effacing *Escherichia coli* Strains from Monkeys

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Typing of Intimin (*eae*) Genes in Attaching and Effacing *Escherichia coli* Strains from Monkeys

Attaching and effacing *Escherichia coli* (AEEC) strains cause histopathological alterations termed "attaching and effacing (A/E) lesions" (8). The ability to cause A/E lesions is encoded on a large bacterial chromosomal pathogenicity island, the locus of enterocyte effacement (LEE). The central portion of LEE encodes intimin (Eae, 94- to 97-kDa outer membrane protein) and Tir, the intimin receptor, which is translocated into the host cell membrane by the type III system (8). Differentiation of intimin alleles represents an important tool for AEEC typing in pathogenesis and epidemiological, clonal, and immunological studies, and it may also be a potential tool in routine diagnostics (1, 2, 3, 4, 12, 15). The 5′ regions of *eae* genes are conserved, whereas the 3′ regions are heterogeneous. This observation led to the construction of universal PCR primers and allele-specific PCR primers, which made it possible to differentiate, at present, 15 variants of the *eae* gene that encode 15 different intimin types and subtypes (2, 4). Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains causing A/E lesions in the intestinal mucosa are considered AEEC (8, 14). In contrast to STEC, EPEC strains do not produce Shiga toxins. EPEC strains are a major cause of infant diarrhea in developing parts of the world and are pathogenic to several animal species (rabbits, calves, dogs, sheep, pigs, and primates) (3, 4, 5, 14). However, the serotypes of human and animal EPEC strains are usually different. Typical human EPEC strains present the *bfp* gene, which encodes the fimbriae called bundle-forming pili (BFP) (7, 14).

Although enteric diseases, specifically diarrhea, are frequently associated with morbidity and mortality in nonhuman primates in captivity, studies of the role of different diarrheagenic *E. coli* strains in these diseases are lacking. Thomson and Scheffler (13) reported an outbreak of diarrhea caused by a Shiga toxin-negative AEEC isolate of serogroup O26 in marmosets maintained at the Primatology Center. Mansfield et al. (9, 10) associated a Shiga toxin-negative AEEC O156:H−, intimin ε-positive strain with a simian immunodeficiency virus opportunistic infection in rhesus monkeys (10) and a Shiga toxin-negative AEEC O26:H− ε intimin-positive strain with ulcerative colitis in cotton-top tamarins (9). Recently, Carvalho et al. (5) found that AEEC strains harboring genes for intimin production (*eae* positive) and lacking genes for Shiga toxin production (*stx1* and *stx2* negative) were the only group of diarrheagenic *E. coli* strains isolated from fecal samples of diarrheic and healthy marmosets. Eighteen of 56 (32%) animals carried *E. coli* strains with the *eae* gene, including 8 of 17 (47%) with diarrhea and/or enteritis and 10 of 39 (26%) healthy animals. All monkey AEEC strains isolated by Carvalho et al. (5) were able to cause the A/E lesion, as determined by the FAS test and confirmed by electron microscopy of infected HEP-2 cells. Monkey AEEC strains isolated by Carvalho et al. (5) were also examined for intimin subtypes α, β, δ, and γ, as described previously (1). Because the number of intimin subtypes studied was very limited, the majority of monkey strains showed nontypeable intimins (5). In order to ascertain whether these intimin subtypes were actually new ones, some of these strains were examined again by PCR using a set of new primers described by Blanco et al. (2, 4) for the already known intimins as well as for new *eae* variants β2, μ, ν, and ξ. For comparison studies, the monkey strains were serotyped by the method described by Guinée et al. (6), and the previous results obtained for *bfp* by PCR, as well as BFP expression by Western blotting, were reconsidered in this study (5).

All 15 monkey *E. coli* strains assayed were positive with universal primers EAE-1 and EAE-2 that generated PCR products obtained from the amplified 5′-conserved region of the *eae* gene. Six monkey AEEC strains presented identical serotypes and intimins (two O142:H6 α1, two O128:H2 β1, and two O127:H40 γ2/θ strains) to human enteropathogenic *E. coli* (EPEC), whereas eight strains showed new serotypes not previously found in human or animal AEEC with β1 (two O132:H31 strains), β2 (one O139:H14 strain and one O167:H6 strain), ε (one O26:H7 strain), ι (two O49:H46 strains), and λ (one O33:H−) intimins. The remaining monkey strain, which belonged to serotype O167:H9 (β1), although it was not included among human EPEC serotypes, was characterized as an AEEC strain that caused an outbreak of gastroenteritis involving a large number (256 patients) of schoolchildren (11) (Table 1). The intimins α2, γ1, δ/κ, ζ, η, μ, ν, and ξ were not found among the AEEC strains isolated from marmosets in Brazil. However, considering that only 15 strains were studied, the

TABLE 1. Serotypes and intimin types of monkey AEEC strains isolated in Brazil

No. of isolates	Status	Serotype	*bfpA* gene/BFP expression	Intimin subtype	Description[a]
1	Diarrhea	O142:H6	+/+	α1	Human EPEC serotype
1	Diarrhea	O142:H6	−/−	α1	Human EPEC serotype
2	1 Diarrea, 1 healthy	O128:H2	−/−	β1	Human EPEC serotype
2	Healthy	O132:H31	+/+	β1	New serotype
1	Healthy	O167:H9	−/−	β1	Human/outbreak
1	Diarrhea	O139:H14	−/−	β2	New serotype
1	Diarrhea	O167:H6	+/−	β2	New serotype
2	1 Diarrhea, 1 healthy	O127:H40	−/−	γ2/θ	Human EPEC serotype
1	Diarrhea	O26:H7	−/−	ε	New serotype
2	Healthy	O49:H46	−/−	ι	New serotype
1	Healthy	O33:H-	−/−	λ	New serotype

[a] New serotype represents a serotype not found in human or animal AEEC with the indicated intimin subtype in previous studies.

diversity of intimins found among these strains was relatively high.

In conclusion, this study indicates that nonhuman primates may represent a natural resevoir of EPEC serotypes pathogenic for humans.

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