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Escherichia coli that Cause Diarrhea: Enterotoxigenic, Enteropathogenic, Enteroinvasive, Enterohemorrhagic, and Enteroadherent

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There are four major categories of diarrheagenic *Escherichia coli:* enterotoxigenic (a major cause of travelers' diarrhea and infant diarrhea in less-developed countries), enteroinvasive (a cause of dysentery), enteropathogenic (an important cause of infant diarrhea), and enterohemorrhagic (a cause of hemorrhagic colitis and hemolytic uremic syndrome). Besides manifesting distinct clinical patterns, these categories of *E. coli* differ in their epidemiology and pathogenesis and in their O:H serotypes. Common features (albeit distinct for each category) include plasmid-encoded virulence properties, characteristic interactions with intestinal mucosa, and elaboration of various types of enterotoxins or cytotoxins. A less-well-defined fifth category of diarrheagenic *E. coli* is that of enteroadherent *E. coli*, so far identifiable only by their pattern of adherence to Hep-2 cells in tissue culture.

As the predominant species among the facultative anaerobic normal flora of the intestine, *Escherichia coli* plays an important role in maintaining intestinal physiology [1]. Within this species, however, there are fully pathogenic strains that cause distinct syndromes of diarrheal disease. To the uninitiated, the plethora of terms and acronyms used to describe the diarrheagenic *E. coli* (table 1) represent a potential source of confusion; it is often difficult to discern which terms are redundant and which represent distinct entities. In this review, I shall attempt to clarify the situation and explain the four main categories of diarrheagenic *E. coli;* the categories are based on distinct virulence properties, different interactions with the intestinal mucosa, distinct clinical syndromes, differences in epidemiology, and distinct O:H serotypes (figure 1). The four main categories of diarrheagenic *E. coli* are (*1*) enterotoxigenic *E. coli* (ETEC), (*2*) enteroinvasive *E. coli* (EIEC), (*3*) enteropathogenic *E. coli* (EPEC), and (*4*) enterohemorrhagic *E. coli* (EHEC).

The EPEC category can be conveniently subdivided into two classes that, on a rational pathogenetic basis, include strains referred to by some authors as attaching and effacing *E. coli* (AEEC) [2]. Class I EPEC exhibit localized adherence to Hep-2 cells, whereas class II EPEC exhibit either diffuse adherence or no adherence at all to Hep-2 cells. There is also a fifth category of diarrheagenic *E. coli*, enteroadherent *E. coli* (EAEC) [3, 4]. Although little is known about the pathogenesis, epidemiology, and serotypes of the strains in this category of *E. coli*, preliminary evidence suggests that they are indeed capable of causing diarrheal disease, and they clearly do not fit into the other four cateogries; therefore, they have been relegated to a fifth category, at least until further information becomes available.

Although the four main categories of diarrheagenic *E. coli* are quite distinct, they nevertheless have certain underlying commonalities with respect to pathogenesis. (*1*) Critical virulence properties are encoded in plasmids. (*2*) There is a characteristic interaction with intestinal mucosa. (*3*) Enterotoxins or cytotoxins are produced. (*4*) Within each category, the strains tend to fall within certain O:H serotypes.

In the 1940s, Kauffman [5] proposed a scheme to differentiate *E. coli* on the basis of lipopolysaccharide O, flagellar H, and polysaccharide K antigens. We presently recognize 171 O and 56 H serogroups. Together, these constitute the O:H system, which has played an important role in studies of the epidemiology and pathogenesis of *E. coli* infection.

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Table 1. Plethora of descriptive terms for *E. coli* associated with diarrhea.

Organism	Acronym
Enterotoxigenic *E. coli*	ETEC
Enteroinvasive *E. coli*	EIEC
Enteropathogenic *E. coli*	EPEC
Classical serotype EPEC	
Traditional serotype EPEC	
Adherent enteropathogenic *E. coli*	
Cytotoxic enteropathogenic *E. coli*	
Enterocyte-adherent enteropathogenic *E. coli*	EAEPEC
Facultative enteropathogenic *E. coli*	FEEC
Attaching and effacing *E. coli*	AEEC
Enteroadherent *E. coli*	EAEC
Enterohemorrhagic *E. coli*	EHEC
Verotoxin-producing *E. coli*	VTEC

Recognition that *E. coli* Cause Diarrhea

In the early 1940s, summer diarrhea was an important clinical problem in infants in Europe and North America. In England at this time, a microbiologist – John Bray – and a pediatrician – John Beavan – decided to study the problem [6, 7]. They were aware that the routine stool cultures in use at that time detected *Shigella* and *Salmonella*, but failed to identify pathogens in most infants. These investigators hypothesized that some strains of *E. coli* that appeared to be strains of normal flora by colonial morphology on agar might actually be pathogenic. To pursue this hypothesis, they used an immunizing strain of *E. coli* isolated from an infant named Wickens who had summer diarrhea – with no other pathogens – to prepare an antiserum that might identify the homologous and related strains of *E. coli*. In an example of productive research despite meager resources, they immunized a pet rabbit named Snowy to obtain the typing serum [8]. This study was carried out before the Kauffman O:H serotyping system for *E. coli* was described.

Bray and Beavan examined cultures of stools from infants with summer diarrhea and from healthy control infants for strains of *E. coli* that could be agglutinated by the rabbit antiserum. Agglutinating *E. coli* were detected in cultures from 92% of 90 diarrheal infants but in only 6% of 180 controls [6, 7]. They referred to this strain as *Bacillus coli* var. *neapolitanum;* it is now known to belong to serogroup O111. After these results were published, investigators in other countries pursued studies by using the same general approach [9–20]. As a consequence, by the mid-1950s, a series of *E. coli* strains had been epidemiologically incriminated throughout the world as important causes of infant diarrhea [21–23]; the term *enteropathogenic E. coli* (EPEC) was proposed by Neter [24] to refer to these serotypes. The major O serogroups considered to contain EPEC serotypes are shown in figure 1; except for O142, this list represents a compilation of reports from Ewing et al. [25] and Taylor [26], who headed the enteric reference laboratories at the Centers for Disease Control (Atlanta) and the Central Public Health Laboratory (Colindale, England), respectively, in the 1950s. O142 was identified as a major EPEC serotype in the late 1960s and early 1970s [27–30]. Only certain H types are incriminated within each O serogroup [25, 26]. I will henceforth refer to these O serogroups as "classical" EPEC O serogroups. Certain other O serogroups were recognized by both Ewing et al. [25] and Taylor [26] as having some incrimination as pathogens, but not to the same extent as the major classical O serogroups. These other O serogroups include O18, O44, O112, and O114, among others (figure 2).

Studies conducted with volunteers during the early 1950s clearly established the pathogenicity of strains from several of the most common classical EPEC O serogroups, including O55, O111 and O127 [31–35]. Nevertheless, although both epidemiological evidence and studies with volunteers established the classical serotype EPEC as causes of diarrhea, it was not possible to distinguish between these *E. coli* and strains of normal flora by the tests and animal models available up to the mid 1960s, and the virulence properties by which they caused diarrhea remained unknown. Therefore, O serogrouping remained the only diagnostic tool for EPEC and was common until the early 1970s. Unfortunately, only

1. ENTEROTOXIGENIC

2. ENTEROINVASIVE

3. ENTEROPATHOGENIC:

CLASS	HEp-2 ADHERENCE	EAF PROBE	SEROTYPES
I	Localized	+	Classical
II	Negative or diffuse	-	Classical

4. ENTEROHEMORRHAGIC

5. ENTEROADHERENT

Figure 1. The distinct categories of diarrheagenic *E. coli*.

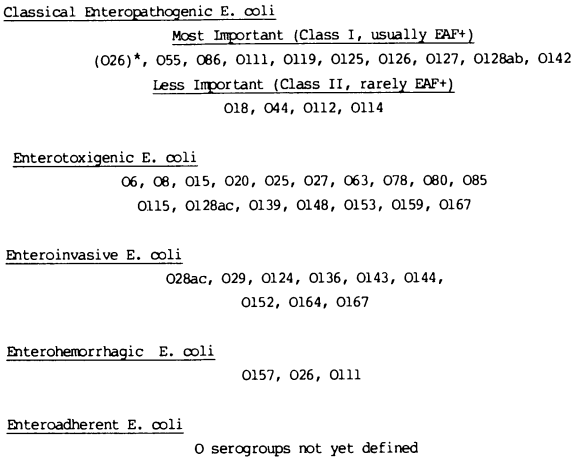

Figure 2. O serogroups associated with the major categories of diarrheagenic *E. coli*. *, Serogroup O26 was originally categorized as an EPEC, but is now considered to be an EHEC. EAEC is a provisional, less-well-defined category of diarrheogenic *E. coli*.

a minority of laboratories had the reagents, techniques, and expertise necessary for proper serogrouping.

ETEC

The second major class of *E. coli* to be associated with diarrhea are ETEC, which came to prominence in the late 1960s and early 1970s, largely on the basis of work carried out in Calcutta by Gorbach, Sack, and co-workers [36, 37]. ETEC are a major cause of infant diarrhea in less-developed countries (some reports of infants prospectively followed-up by frequent household surveillance suggest that as many as two to three clinical ETEC infections per child per year occur during the first two to three years of life) [38], one of the main bacterial causes of dehydrating infant diarrhea in developing areas [39], and an infection correlated with adverse nutritional consequences [40]. ETEC are also the agent most frequently responsible for travelers' diarrhea [41–43]. ETEC diarrhea within the United States and other developed countries is rare, although occasional outbreaks have been reported [44, 45].

ETEC infection is acquired by ingesting contaminated food or water. The bacteria colonize the proximal small intestine, the critical site of host-parasite interactions, where they elaborate heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST). The reader is referred elsewhere for reviews of the properties of LT and ST [46]. The clinical features of ETEC infection are watery diarrhea, nausea, abdominal cramps, and low-grade fever.

When collections of ETEC from diverse geographic areas were serotyped, a limited number of O:H serotypes occurred repeatedly throughout the world and accounted for a majority of the ETEC strains [47–55]. Although many other serotypes can also be toxigenic, the recurrent O:H serotypes appear to be successful ETEC clones that have spread far and wide. Usually, these serotypes elaborate both LT and ST and possess fimbrial colonization factors [52–55]. The major O serogroups associated with ETEC are shown in figure 2.

Adherence of ETEC. ETEC possess attachment or colonization factors that allow them to overcome the peristaltic defense mechanism of the small intestine. Heretofore, all the characterized colonization factors have been fimbriae – hair-like, filamentous organelles (notably thinner than flagellae) on the surface of the *E. coli*. The fimbrial colonization factors of human ETEC have certain features in common [46]. (*1*) They consist of either rigid structures 6–7 nm in diameter or wiry, flexible structures 2–3 nm in diameter. (*2*) They are encoded by plasmids that almost always also encode ST and often LT as well. (*3*) They consist of protein subunits 14–22 kilodaltons (kDa) in size. (*4*) In the presence of mannose, many, but not all, fimbrial colonization factors mediate hemagglutination of certain erythrocytes (a characteristic differentiating them from type 1 fimbriae). (*5*) Fimbrial colonization factors are expressed in cultures grown at 37 C, but not at 18 C. (*6*) Particular colonization fimbriae are largely restricted to certain O:H serotypes.

There has been keen interest in the variety of fimbrial colonization factors present in human ETEC, because this information is directly relevant to the polyvalency of an anti-colonization factor vaccine against ETEC and to the breadth of protection that might be expected from such a vaccine [46].

The first fimbrial colonization factor in a human ETEC strain, described by Evans et al. [56, 57] in the mid 1970s and referred to as CFA/I, was found in ETEC of serotypes O15, O25, O63, O78, and O128 (table 2). Evans et al. reported that the presence of CFA/I could be suspected on the basis of a particular hemagglutination pattern and confirmed by using antiserum to CFA/I. Several other laboratories substantiated these findings.

Evans et al. [58] reported a second, antigenically

Table 2. Relation of O serogroup to colonization factor fimbriae in enterotoxigenic *E. coli*.

Fimbrial antigen	O serogroup(s)
CFA/I	15, 25, 63, 78, 128, 153
CFA/II	
CS1	6, 139
CS2	6
CS3	6, 8, 80, 85, 139
E8775	
CS4	25
CS5	92, 115
CS6	25, 27, 92, 115, 148, 169
PCF0159	159

distinct fimbrial colonization factor, referred to as CFA/II, that was associated with serogroups O6, O8, O80, and O85; CFA/II manifested a different hemagglutination pattern than did CFA/I. As other investigators followed the lead of Evans, it became clear that the situation with CFA/II was much more complicated than that with CFA/I. The initial breakthrough in clarifying this situation was made, essentially simultaneously, by Cravioto et al. [59] and Smyth [60]. Cravioto et al. showed that strains tentatively identified as CFA/II on the basis of hemagglutination patterns were in fact elaborate combinations of three distinct antigens, which they called components 1, 2, and 3. Smyth made identical observations and referred to the antigens as CS1, CS2, and CS3 (table 2). CS3 was present in virtually all the strains. However, depending on the serotype and biotype, they observed strains expressing CS1 and CS3, CS2 and CS3, or CS3 only. Strains simultaneously expressing both CS1 and CS2 were never found. Smyth [60] reported the subunit sizes of the antigens as 16.3 (CS1), 15.3 (CS2), and 14.7 kDa (CS1), and Smith et al. [61] reported that a single plasmid encodes the genes for CS1, CS2, and CS3 and that the determination of which fimbrial antigens are expressed is a function of the *E. coli* host bacteria and is related to serotype and biotype.

Both Mullaney et al. [62] and Smyth [63] described CS1 and CS2 as typical 6–7-nm diameter, rigid fimbriae that resembled CFA/I in morphology. However, they reported that CS3, the antigen common to virtually all CFA/II strains, was not fimbrial; instead, CS3 was described as amorphous and without detectable structure upon examination by electron microscopy.

Working with strains expressing both CS1 and CS3, as well as with strains expressing only CS3 in vitro, Levine et al. [64] and Knutton et al. [65] identified thin, wiry, flexible structures 2–3 nm in diameter that appeared in electron photomicrographs; these structures were shown to be CS3. The identification of these flexible fibrillae as CS3 was confirmed by purifying the antigen, by showing the specificity of its reactivity with antibody to CS3 by immunodiffusion and immunoblotting techniques, and by immunolabeling the structures with colloidal gold and visualizing them under the electron microscope [64].

These observations showed for the first time that, in addition to rigid 6–7-nm fimbrae such as CFA/I, human ETEC pathogens have flexible, 2–3-nm fibrillar-type fimbriae that resemble K-88 and F41 antigens of porcine strains [66, 67].

Clarifying the CFA/II family of antigens provided important basic information but did not expand the number of O serogroups that could be correlated with the presence of known colonization fimbriae. However, at approximately the same time, Thomas et al. [68] described a new putative colonization factor fimbria in prototype strain E8775. This new colonization factor was found in ETEC of serogroup O25, and for the first time in O115 and O167. More recently, these authors have shown that E8775, like CFA/II, consists of a family of three distinct antigens [69]. Two of these, CS4 and CS5, are rigid 6–7-nm fimbriae, whereas CS6, like CS3, is not. Furthermore, they have identified CS4, CS5, and CS6 in several of the ETEC O serogroups for which there previously had been no recognized colonization antigen, including the very common serogroups O27 and O148 (table 2).

The last of the important ETEC O serogroups not associated with a known colonization factor fimbria was O159 (table 2). Tacket et al. [70] have solved this problem. In contrast with other ETEC strains associated with colonization factor fimbriae, O159:H4 strains do not manifest mannose-resistant hemagglutination. Nevertheless, it was found that SDS-PAGE of disaggregated sheared preparations of an O159:H4 strain grown at 37 C revealed a distinct 19-kDa subunit band that was not visible in sheared preparations from cultures grown at 18 C [70]. This band apparently corresponded to a nonhemagglutinating fimbria. Tacket et al. [70] visualized this fimbria, purified it, showed that it is encoded on a 27-megadalton (MDa) plasmid that also encodes LT and ST, demonstrated that it is antigenically distinct from other colonization factor fimbriae, and showed

that it is present on O159:H4 strains from diverse parts of the world.

Several other putative colonization factors have been reported [71–73], some of which may share antigenic identity with the previously described factors. It is not known if the fimbria described by Honda et al. [72] is encoded on plasmids that also encode LT or ST (which would add credibility to its being a colonization factor), nor with what serotypes it is associated. The identity of the antigens described by Deneke et al. [71] and whether they cross-react with CFA/I, CS1–CS6, or PCFO159 is not clear. A recent antigen described by Darfeuille et al. [73] is probably CS6, as evidenced by the serotype of the prototype strain, the size of the subunit, and the fact that it is not a rigid fimbrial structure.

Thus, colonization factor antigens have now been identified in all the main O serogroups associated with ETEC. An obvious related question is what proportion of human ETEC pathogens possess known colonization factor fimbriae. There has been debate on this point. Two recent studies from Asia suggest that the proportion is quite high [74, 75], particularly if fresh isolates are examined. Heretofore, no study has tested strains for all the fimbrial antigens described in this review. At least one such study will be initiated shortly in South America.

Considerable work is underway on the development of candidate oral vaccines to prevent ETEC diarrhea. Some of the suggested vaccines include purified CFA fimbriae, LT/ST toxoid, killed fimbriate *E. coli*, or attenuated strains of *E. coli* (A^-B^+, fimbriated, ST toxoid) or *Salmonella typhi* (modified Ty21a or 541Ty). Evans et al. [76], and Levine et al. [77, 78], previously worked with purified fimbriae; this approach has been largely abandoned. Klipstein et al. [79] have recently reported the oral administration of a synthetic LT/ST toxoid to volunteers; it was well-tolerated and stimulated serum and intestinal antibodies to LT and ST. Killed, fimbriated *E. coli* are also being explored as an oral vaccine. Lastly, live oral vaccines are being developed. Clements and El-Morshidy [80] and Yamamoto et al. [81] have described the use of attenuated *Salmonella typhi* strain Ty21a as a carrier bacteria to express introduced genes encoding putative protective antigens of ETEC. Levine et al. [78, 79] have explored the use of nontoxigenic *E. coli* expressing fimbrial colonization factor antigens as live oral vaccines.

A group of volunteers were given a single oral dose of 10^{10} organisms of strain E1392/75-2A; this strain is serotype O6:H16 and bears CS1 and CS3 fimbriae, but lacks the genes encoding LT and ST [78, 79]. Ten recipients of the vaccine, from whom paired prevaccination and postvaccination samples of intestinal fluids were obtained, showed significant rises in titer of antibody to fimbriae (prevaccination geometric mean titer, 5; postvaccination geometric mean titer, 416); the secretory IgA response was much greater than that observed in persons who received three evenly spaced enteral doses of purified fimbriae. The vaccinees were challenged one month later, along with a group of unimmunized control volunteers, with a toxigenic strain of a different O:H serotype bearing CS1 and CS3 fimbriae. All controls but only three of 12 vaccinees developed diarrhea, a highly significant difference; furthermore, illness in the three vaccinees was milder. Bacteriologic studies showed that the mechanism of protection was prevention of colonization of the proximal small intestine. Duodenal cultures were positive in five of six controls (mean, 7,000 organisms/ml) versus only one of 12 vaccinees (mean, 10 organisms/ml; $P < .004$).

EIEC

In 1971, DuPont et al. [82] described certain *E. coli* strains that caused an invasive, dysenteric form of diarrheal illness in volunteers. These strains, of serotypes distinct from ETEC and EPEC (figure 2), closely resembled *Shigella* in many ways. Like *Shigella*, their cardinal pathogenetic feature is the capacity to invade and proliferate within epithelial cells and cause eventual death of the cell [82]. The invasive capacity of both EIEC and *Shigella* is dependent on the presence of large (~140 MDa) plasmids [83] coding for the production of several outer membrane proteins involved in invasiveness [84]; the proteins are antigenically closely related (if not identical) in EIEC and in *Shigella*. EIEC often resemble *Shigella* in being nonmotile and unable to ferment lactose. Furthermore, EIEC and *Shigella* O antigens show many cross-reactions.

EIEC have a predilection for colonic mucosa as the favored site of host-parasite interaction. Clinically, the illness is marked by fever, severe abdominal cramps, malaise, toxemia, and watery diarrhea followed by gross dysentery consisting of scanty stools of blood and mucus. A simple stain of the fecal mucus reveals sheets of PMNLs.

EIEC can be diagnosed by serotyping suspect *E. coli* strains [85], by an ELISA that detects the

outer membrane proteins associated with invasiveness [86], and by DNA probes that detect the genes for invasiveness [87].

EPEC

By the mid 1970s, the properties of LT, ST, and *Shigella*-like invasiveness in *E. coli* strains from certain patients with diarrhea (e.g., travelers) was well-established, as was the fact that these strains were only rarely of the classical EPEC O:H serotypes that had been epidemiologically incriminated in the 1950s as causes of infant diarrhea [88–94]. Conversely, several investigators examined stored EPEC strains from several outbreaks of infant diarrhea and recent isolates from patients with infant diarrhea for the presence of LT, ST, and epithelial cell invasiveness [90, 91, 93]; with rare exceptions, the strains lacked these properties. This precipitated a lively controversy. Some investigators concluded that EPEC were not pathogens because they did not manifest the known ETEC or EIEC properties of virulence, and they argued that in these strains the critical toxin or invasiveness plasmids were unstable and had been lost during subculture and storage [90–92, 95]. On the basis of epidemiological evidence, other investigators concluded that the classical EPEC strains were obviously pathogens, but caused diarrhea by mechanisms distinct from LT, ST, and *Shigella*-like invasiveness [89].

At the Center for Vaccine Development (University of Maryland School of Medicine, Baltimore), an attempt was made in 1977 to resolve this controversy by studying volunteers challenged with several EPEC strains lacking LT, ST, and invasiveness; these strains had been stored for up to seven years after isolation during outbreaks of infant diarrhea [97]. When these strains were fed to healthy young adult volunteers, they caused definite diarrhea (one individual developed severe, cholera-like illness and purged 5.6 liters of watery diarrhea). Therefore, EPEC were indeed pathogens, but they caused diarrhea by mechanisms unknown at that time. This study also stimulated renewed research on the pathogenesis of EPEC diarrhea and rapidly yielded results.

Polotsky et al. [98] noted that EPEC strains cause a distinctive ultrastructural histopathologic lesion in human intestines (visible by electron microscopy) that is not seen with ETEC strains. The distinctive lesion involves destruction of the microvilli by the EPEC bacteria, typically without further evidence of invasion. The bacteria are often closely adherent to the membrane of the enterocyte, with the membrane often cupping or partially enveloping the bacterium [99]. This same ultrastructural lesion was later described in biopsies from infants with classical serogroup O125 and O119 EPEC infections by Ulshen and Rollo [100] and Rothbaum [101]. This lesion has been studied in gnotobiotic piglets by Moon et al. [2] and Tzipori et al. [102].

In another hallmark study, Cravioto et al. [103] observed that approximately 80% of the EPEC strains in the Colindale Laboratory collection adhered to Hep-2 cells in tissue culture, a property rare in ETEC, EIEC, or normal flora strains. Shortly thereafter, Baldini et al. [104] reported that 30 of 31 classical serotype EPEC strains from patients with diarrhea possess a plasmid ~60 MDa in size that encodes the property of adhesiveness to Hep-2 cells described by Cravioto; the name EPEC adherence factor (EAF) was given to this property. Later, Scaletsky et al. [105] and Nataro et al. [106] clearly differentiated localized adherence to Hep-2 and HeLa cells from diffuse adherence and showed that the former was plasmid-mediated.

Levine et al. [107] carried out a study to determine the role of the EAF plasmid in causing EPEC diarrhea in volunteers. Nine of 10 volunteers who ingested an O127:H6 strain with the EAF plasmid developed definite diarrhea; in contrast, only two of nine volunteers who ingested an EAF-negative variant of the strain developed diarrhea, and it was extremely mild ($P < .006$).

Preparations of the outer membranes of the O127 strain with and without its plasmid (compared by SDS-PAGE) revealed a prominent plasmid-encoded protein of 94-KDa [107]. By means of an immunoblotting technique, it was found that recipients of the EAF-positive strain made antibody to the 94-KDa protein in response to infection [107]. Furthermore, the one individual among the 10 challenged who failed to develop diarrhea was the only one who had antibody against this protein before challenge, a result suggesting that he was immune and that this protein may be a critical protective antigen. By using antiserum specific for the 94-KDa protein, this protein has been found in all the important EPEC serotypes, such as those in serogroups O55, O111, O119, O127, and O142. In contrast, this 94-KDa protein has not been found in ETEC, EIEC, EHEC, or in strains of *E. coli* that cause meningitis or pye-

lonephritis. An attempt to purify the 94-KDa protein is underway to prepare a potent and plentiful antibody that can be used in a simple diagnostic test, such as one based on agglutination.

In the meantime, an excellent test to identify EPEC already exists. Nataro et al. [108] have cloned a 1-kilobase segment of DNA from the EAF plasmid of strain E2348/69, and they have shown it to be a highly sensitive and specific DNA probe for detecting EPEC that carry the EAF plasmid. In a collaboration with Valeria Prado of Santiago, Chile, we found that the EAF probe hybridized with approximately 75% of *E. coli* strains identified as suspect EPEC by multi-step O serogrouping carried out by Dr. Prado's laboratory (unpublished data). More recently, the question has arisen as to whether *E. coli* other than recognized EPEC serotypes also carry the EAF plasmid and whether they are diarrheagenic. This has been answered in a recent study carried out by Levine et al. (unpublished data) in collaboration with Dr. Valeria Prado in Santiago, Chile. In 11 (31%) of the 47 infants from whom EAF-positive *E. coli* were recovered, the *E. coli* were of non-EPEC serogroups. However, whereas EAF-positive *E. coli* of classical serogroups were recovered significantly more frequently from infected patients (35 [23%] of 154) than from controls (1 [2%] of 66; $P = .0002$), EAF-positive strains of nonclassical serogroups were recovered with comparable frequency from infected patients (7 [5%] of 154) and controls (4 [6%] of 66). Thus, on the basis of preliminary epidemiological evidence from Chile, only EAF-positive *E. coli* of classical EPEC O serogroups appear to be capable of causing diarrhea. Presumably this is true because other virulence properties are required, and these accessory virulence properties appear to be restricted to classical serogroup strains.

EPEC serotypes that do not possess the EAF plasmid and do not give localized adherence to Hep-2 cells have also been incriminated by epidemiological studies and studies in volunteers as causes of diarrhea, even though their pathogenesis at the molecular level is not as precisely known as that for the EAF-positive EPEC. Nevertheless, at least for the present, these EAF-negative EPEC will be considered as a second class (class II) within the EPEC category. One such strain of classical serotype O114:H2 possesses two plasmids of ~60 MDa, exhibits no adherence to Hep-2 cells, and causes definite diarrhea. Moon et al. [2] found that this strain caused typical attaching and effacing lesions in enterocytes that were indistinguishable from those caused by EAF-positive EPEC. Thus, for both historical and pathological reasons, we have elected to refer to these strains as Class II EPEC [108], i.e., EAF-negative strains of classical EPEC serotypes (figure 2).

Marques et al. [109] and Cleary et al. [110] have reported that some EPEC strains elaborate moderate quantities of a cytotoxin very similar (or identical) to the toxin from *Shigella dysenteriae* type 1. It has been suggested that this toxin may play a role in the pathogenesis of EPEC disease. As I will show below, many of the cytotoxigenic strains referred to as EPEC serogroups and serotypes by Marques et al. and Cleary et al. should be more correctly categorized as enterohemorrhagic. In particular, this is true of O26:H11, which was heretofore considered a classical EPEC serotype.

Clinically, EPEC illness is characterized by fever, malaise, vomiting, and diarrhea with prominent amounts of mucus but without gross blood. EPEC illness tends to be clinically more severe than many other diarrheal infections in infants, some of whom develop prolonged diarrhea that persists for >14 days. Recent studies from several countries in South America have shown EPEC to be either the first or second most important bacterial cause of diarrhea in infants [111–113].

EAEC

The other diarrheagenic *E. coli* of interest are the EAF-negative, nonclassical serotype strains described by Mathewson [3, 4] and identified only by their property of adherence to Hep-2 cells. After their incrimination in an epidemiological study [3], challenge of volunteers showed that at least one strain caused mild but definite diarrhea without blood or fecal leukocytes [4]. Preliminary evidence suggests that these *E. coli* are identifiable by a particular pattern of adherence to Hep-2 cells that is clearly distinguishable from both localized adherence and diffuse adherence (J.P. Nataro et al., unpublished observations; M. M. L. et al., unpublished observations). EAEC do not elaborate LT, ST, or elevated levels of Shiga-like toxin, invade epithelial cells, or possess EAF plasmids. It is critical to determine whether the pathogenicity of EAEC and their adherence to Hep-2 cells is related to certain plasmids and whether they fall within a restricted set of O serogroups, as has been found with the other cate-

gories of diarrheagenic *E. coli*. It is also important to determine the relation, if any, between Mathewson's strains and the fimbria-mediated diffuse adherence described by Moseley et al. [114]. For the moment, at least, I suggest that the strains described by Mathewson et al. be considered a distinct, fifth category of diarrheagenic *E. coli*. Further data, however, must be generated before these strains can be fully accepted as diarrheagenic *E. coli*. One other epidemiological study carried out in Mexico [115] failed to identify diffuse Hep-2 cell–adherent *E. coli* any more commonly in children with diarrhea than in controls. It is very possible that this last, less-well-defined category is heterogeneous.

EHEC

In 1982, a multistate outbreak of hemorrhagic colitis drew attention to an unusual clinical syndrome of diarrheal disease and a new bacterial enteric pathogen [116]; the causative organism, *Escherichia coli* O157:H7, was a serotype not previously recognized as a cause of diarrheal disease in humans. The clinical syndrome was notable in that bloody but copious diarrhea, unaccompanied by fecal leukocytes, was seen in afebrile patients [116]; these features distinguish it from classic dysentery due to *Shigella* or EIEC, infections that are characterized by fever and scanty stools of blood and mucus containing many fecal leukocytes [82]. Since 1982, some knowledge has been gleaned on the epidemiology of O157:H7 infections as they occur in North America and Europe, and considerable progress has been made on elucidating its pathogenesis. There has also been a strong incrimination of O157:H7 as a cause of hemolytic uremic syndrome [117–124]. O157:H7 has emerged as an enteric pathogen of public health importance in Canada and the United States, with multiple reports of outbreaks of hemorrhagic colitis, hemolytic uremic syndrome, and diarrhea in nursing homes, day care centers, schools, and the community [117–129].

O157:H7 strains from persons with hemorrhagic colitis and hemolytic uremic syndrome elaborate phage-encoded potent cytotoxins active on HeLa and Vero cells [129–136]. One of these toxins, *Shiga-like toxin 1* or *Verotoxin 1*, is apparently identical to the potent cytotoxin/neurotoxin/enterotoxin produced by *S. dysenteriae* type 1 (Shiga toxin) [137, 138] and reacts with and is neutralized by antibody to Shiga toxin. Many strains also elaborate a second potent cytotoxin (Shiga-like toxin 2 or Verotoxin 2) that is not neutralized by antibody to Shiga toxin [130, 133, 135, 136]. In addition, O157:H7 strains possess a 60-MDa plasmid that plays a role in virulence. Recently, Karch et al. [139] have shown that this plasmid encodes the production of a newly recognized variety of fimbriae that appears to mediate attachment to Henle 407 gut-derived epithelial cells in tissue culture. They examined 14 O157:H7 strains isolated from patients with hemorrhagic colitis or hemolytic uremic syndrome for their plasmid content and for the presence of fimbriae. Thirteen of the strains possessed a 60-MDa plasmid and were fimbriated [139]. Three strains cured of the 60-MDa plasmid lacked fimbriae. Conversely, a nonfimbriated K-12 strain expressed fimbriae after being transformed with the 60-MDa plasmid from O157. A tissue culture assay with Henle 407 cells showed that O157:H7 bacteria containing the 60-MDa plasmid adhere to the cells in a characteristic way: small numbers of bacteria (usually two to four) attach in a central location, whereas plasmid-cured derivatives fail to adhere [139].

Several animal models have been developed to demonstrate the pathological features of O157:H7 infection [140–146]. In electron photomicrographs by Tzipori et al. [145], attached and effaced enterocytes are evident with destruction of the microvilli, a lesion resembling that due to classical serotype EPEC [2, 102]. Nevertheless, in gnotobiotic piglets, EHEC and EPEC infections can be clearly differentiated by anatomic site of involvement, severity of lesions, and degree of polymorphonuclear cell infiltration. EPEC involve the entire intestine of piglets, EHEC only the cecum and colon; EPEC lesions are generally less severe; some infiltration by leukocytes is seen with EPEC, but not with EHEC, infection.

The term EHEC refers to strains such as O157:H7, which manifest the above-mentioned clinical, epidemiological, and pathogenetic features.

Heretofore, it has been difficult to undertake studies of the epidemiology of EHEC infections, other than investigations of outbreaks, because of the lack of suitable methods for screening large numbers of stool cultures for O157:H7 strains, the lack of knowledge regarding what other serotypes may also be enterohemorrhagic, and an inability to identify these serotypes. We contend that one other serotype, in particular, O26:H11, should be classified as EHEC; this serotype is usually an abundant producer of verotoxin [147–151], possesses a 60-MDa

plasmid [149, 152–154], does not hybridize with the EAF gene probe from EPEC [108], and is sometimes associated with bloody diarrhea. Recognizing the importance of plasmids in encoding critical virulence properties in EPEC, EIEC, and ETEC and the usefulness of diagnostic DNA probes prepared from these plasmids, we undertook studies at the Center for Vaccine Development to compare the 60-MDa plasmids of O157:H7 and O26:H11 strains, on the hypothesis that the latter are also EHEC. These preliminary studies showed homology among the plasmids and led to the development of a sensitive and specific DNA probe to identify EHEC (M. M. L. et al., unpublished observations).

From the early days of the 1940s when *E. coli* were first convincingly associated with human diarrhea, much has been discovered about the several categories of diarrheagenic *E. coli*, including information on their clinical features, epidemiology, O:H serotypes, and most particularly, their pathogenesis. From a previous state of some confusion about their role as enteric pathogens, diarrheagenic *E. coli* are now recognized as being among the best understood bacterial enteropathogens.

References

1. Drasar BS, Hill MJ. Human intestinal flora. The distribution of bacterial flora in the intestine. London: Academic Press, 1974:36–43
2. Moon HW, Whipp SC, Argenzio RA, Levine MM, Giannella RA. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infect Immun 1983;**41**:1340–51
3. Mathewson JJ, Johnson PC, DuPont HL, Morgan DR, Thornton SA, Wood LV, Ericsson CD. A newly recognized cause of travelers' diarrhea: enteroadherent *Escherichia coli*. J Infect Dis 1985;**151**:471–5
4. Mathewson JJ, Johnson PC, DuPont HL, Satterwhite TK, Winsor DK. Pathogenicity of enteroadherent *Escherichia coli* in adult volunteers. J Infect Dis 1986;**154**:524–7
5. Kauffmann F. The serology of the coli group. J Immunol 1947;**57**:71–100
6. Bray J. Isolation of antigenically homogeneous strains of *Bact. coli neapolitanum* from summer diarrhoea of infants. J Pathol 1945;**57**:239–47
7. Bray J, Beavan TED. Slide agglutination of *Bacterium coli* var. *neapolitanum* in summer diarrhoea. J Pathol 1948; **60**:395–401
8. Bray J. Bray's discovery of pathogenic *Esch. coli* as a cause of infantile gastroenteritis. Arch Dis Child 1973;**48**:923–6
9. Giles C, Sangster GB. An outbreak of infantile gastroenteritis in Aberdeen. J Hyg (Lond) 1948;**46**:1–9
10. Giles C, Sangster G, Smith J. Epidemic gastro-enteritis of infants in Aberdeen during 1947. Arch Dis Child 1949; **24**:45–53
11. Taylor J, Powell BW, Wright J. Infantile diarrhoea and vomiting. A clinical and bacteriological investigation. Br Med J Clin Res 1949;**2**:117–25
12. Kauffmann F, Dupont A. *Escherichia* strains from infantile epidemic gastro-enteritis. Acta Pathol Microbiol Immunol Scand 1950;**27**:552–64
13. Modica RI, Ferguson WW, Ducey EF. Epidemic infantile diarrhea associated with *Escherichia coli* 111, B_4. J Lab Clin Med 1952;**39**:122–8
14. Buttiaux R, Nicolle P, Le Minor L et S, Le Minor S, Gaudier B. Études. Sur les *E. coli* de gastro-entérite infantile. III. Étude épidémiologique dans un service hospitalier du nord de la France. Ann Inst Pasteur 1956;**91**:799–809
15. Braun OH, Specht H. Beitrag zur Epidemiologie der infektiösen Colienteritis der Säuglinge. Eur J Pediatr 1955; **76**:197–207
16. Olitzki AL. Investigations of infantile gastroenteritis in Jerusalem 1953–1956. Acta Medica Orientalia 1956;**15**: 137–44
17. Ørskov F. On the occurrence of *E. coli* belonging to O group 26 in cases of infantile diarrhoea and white scours. Acta Pathol Microbiol Immunol Scand 1951;**29**:373–8
18. Smith J. Association of serological types of *Bacterium coli* with infantile gastroenteritis. J Pathol 1953;**66**:503–11
19. Taylor J, Charter RE. *Escherichia coli* O.128 causing gastroenteritis of infants. J Clin Pathol 1955;**8**:276–81
20. Cooper ML, Walters EW, Keller HM. Detection of a new serotype of *Escherichia coli*, *E. coli* O127:B8, associated with acute diarrhea in infants. J Bacteriol 1955;**69**:689–94
21. Ewing WH, Tatum HW, Davis BR. The occurrence of *Escherichia coli* serotypes associated with diarrheal disease in the United States. Public Health Laboratory 1957;**15**: 118–38
22. Ewing WH. Enteropathogenic *Escherichia coli* serotypes. Ann NY Acad Sci 1956;**66**:61–70
23. Neter E. Enteritis due to enteropathogenic *Escherichia coli*. Present-day status and unsolved problems. J Pediatr 1959;**55**:223–39
24. Neter E. Westphal O, Luderitz O, Needell MH. Demonstration of antibodies against enteropathogenic *Escherichia coli* in sera of children of various ages. Pediatrics 1955;**16**:801–8
25. Ewing WH, Davis BR, Montague TS. Studies on the occurrence of *Escherichia coli* serotypes associated with diarrheal disease. Atlanta, GA: US Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, 1963
26. Taylor J. Host specificity and enteropathogenicity of *Escherichia coli*. J Appl Bacteriol 1961;**3**:316–25
27. Olarte J, Ramos-Alvarez M. Epidemic diarrhea in premature infants. Etiological significance of a newly recognized type of *Escherichia coli* (O142:K86[B]:H6). Am J Dis Child 1965;**109**:436–8
28. Hone R, Fitzpatrick S, Keane C, Gross RJ, Rowe B. Infantile enteritis in Dublin caused by *Escherichia coli* O142. J Med Microbiol 1973;**6**:505–10
29. Love WC, Gordon AM, Gross RJ, Rowe B. Infantile gastroenteritis due to *Escherichia coli* O142. Lancet 1972; **2**:355–7
30. Kennedy DH, Walker GH, Fallon RJ, Boyd JF, Gross RJ, Rowe B. An outbreak of infantile gastroenteritis due to *E. coli* O142. J Clin Pathol 1973;**26**:731–7

31. Ferguson WW, June RC. Experiments on feeding adult volunteers with *Escherichia coli* 111, B_4, a coliform organism associated with infant diarrhea. American Journal of Hygiene 1952;**55**:155–69
32. June RC, Ferguson WW, Worfel MT. Experiments in feeding adult volunteers with *Escherichia coli* 55, B_5, a coliform organism associated with infant diarrhea. American Journal of Hygiene 1953;**57**:222–36
33. Koya G, Kosakai N, Kono M, Mori M, Fukasawa Y. Observations on multiplication of *Escherichia coli* O-111 B_4 in the intestinal tract of adult volunteers in feeding experiments: intubation study with Miller-Abbott's double lumen tube. Jpn J Med Sci Biol 1954;**7**:197–203
34. Koya G, Kosakai N, Fukasawa Y. Supplementary studies on the multiplication of *Escherichia coli* O-111 B_4 in intestinal tract of adult volunteers and its relation to manifestation of coli enteritis. Jpn J Med Sci Biol 1954;**7**:655–61
35. Wentworth FH, Brock DW, Stulberg CS, Page RH. Clinical, bacteriological, and serological observations of two human volunteers following ingestion of *Escherichia coli* O127:B8. Proc Soc Exp Bil Med 1956;**91**:586–8
36. Gorbach SL, Banwell JG, Chatterjee BD, Jacobs B, Sack RB. Acute undifferentiated human diarrhea in the tropics. I. Alterations in intestinal microflora. J Clin Invest 1971;**50**:881–9
37. Sack RB. Enterotoxigenic *Escherichia coli*: Identification and characterization. J Infect Dis 1980;**142**:279–86
38. Black RE, Brown KH, Becker S, Abdul Alim ARM, Huq I. Longtudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhea and association with known pathogens. Am J Epidemiol 1982;**115**:315–24
39. Black RE, Merson MH, Huq I, Alim ARMA, and Yunus M. Incidence and severity of rotavirus and *Escherichia coli* diarrhoeal in rural Bangladesh. Lancet 1981;**1**:141–3
40. Black RE, Brown KH, Becker S. Effects of diarrhea associated with specific enteropathogens on the growth of children in Bangladesh. Pediatrics 1984;**73**:799–805
41. Merson MH, Morris GK, Sack DA, Wells JG, Feeley JC, Sack RB, Creech WB, Kapikian AZ, Gangarosa EJ. Travelers' diarrhea in Mexico. A prospective study of physicians and family members attending a congress. N Engl J Med 1976;**294**:1299–1305
42. Sack DA, Kaminsky DC, Sack RB, Itotia JN, Arthur RR, Kapikian AZ, Ørskov F, Ørskov I. Prophylactic doxycycline for travelers' diarrhea. Results of a double-blind study of Peace Corps volunteers in Kenya. N Engl J Med 1978;**298**:758–63
43. DuPont HL, Olarte J, Evans DG, Pickering LK, Galindo E, and Evans DJ. Comparative susceptibility of Latin American and United States students to enteric pathogens. N Engl J Med 1976;**295**:1520–1
44. Rosenberg ML, Koplan JP, Wachsmuth IK, Wells JG, Gangarosa EJ, Guerrant RL, Sack DA. Epidemic diarrhea at Crater Lake from enterotoxigenic *Escherichia coli*. A large waterborne outbreak. Ann Intern Med 1977;**86**:714–8
45. Ryder RW, Wachsmuth IK, Buxton AE, Evans DG, DuPont HL, Mason E, Barrett FF. Infantile diarrhea produced by heat-stable enterotoxigenic *Escherichia coli*. N Engl J Med 1976;**295**:849–53
46. Levine MM, Kaper JB, Black RE, Clements ML. New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. Microbiol Rev 1983; **47**:510–50
47. Black RE, Merson MH, Rowe B, Taylor PR, Abdul Alim ARM, Gross RJ, Sack DA. Enterotoxigenic *Escherichia coli* diarrhoea: acquired immunity and transmission in an endemic area. Bull WHO 1981;**59**:263–8
48. Echeverria P, Ørskov F, Ørskov I, Plianbangchang D. Serotypes of entertoxigenic *Escherichia coli* in Thailand and the Philippines. Infect Immun 1982;**36**:851–6
49. Merson MH, Ørskov F, Ørskov I, Sack RB, Huq I, Koster FT. Relationship between enterotoxin production and serotype in enterotoxigenic *Escherichia coli*. Infect Immun 1979;**23**:325–9
50. Merson MH, Rowe B, Black RE, Huq I, Gross RJ, Eusof A. Use of antisera for identification of enterotoxigenic *Escherichia coli*. Lancet 1980;**2**:222–4
51. Merson MH, Sack RB, Kibriya AKMG, Abdullah-Al-Mahmood, Adamed QS, Huq I. Use of colony pools for diagnosis of enterotoxigenic *Escherichia coli* diarrhea. J Clin Microbiol 1979;**9**:493–7
52. Ørskov F, Ørskov I, Evans Jr DJ, Sack RB, Sack DA, Wadström T. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. Med Microbiol Immunol (Berl) 1976;**162**:73–80
53. Ørskov I, Ørskov F. Special O:K:H: serotypes among enterotoxigenic *E. coli* strains from diarrhea in adults and children. Occurrence of the CF (colonization factor) antigen and of hemagglutinating abilities. Med Microbiol Immunol (Berl) 1977;**163**:99–110
54. Reis MHL, Matos DP, Pestana de Castro AF, Toledo MRF, Trabulsi LR. Relationship among enterotoxigenic phenotypes, serotypes, and sources of strains in enterotoxigenic *Escherichia coli*. Infect Immun 1980;**28**:24–7
55. Levine MM, Ristaino P, Sack RB, Kaper JB, Ørskov F, Ørskov I. Colonization factor antigens I and II and type 1 somatic pili in enterotoxigenic *Escherichia coli*: relation to entertoxin type. Infect Immun 1983;**39**:889–97
56. Evans DG, Silver RP, Evans Jr DJ, Chase DG, Gorbach SL. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect Immun 1975;**12**:656–67
57. Evans DG, Evans Jr DJ, Tjoa WS, DuPont HL. Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. Infect Immun 1978;**19**:727–36
58. Evans DG, Evans Jr DJ. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups 06 and 08. Infect Immun 1978;**21**:638–47
59. Cravioto A, Scotland SM, Rowe B. Hemagglutination activity and colonization factor antigens I and II enterotoxigenic and non-enterotoxigenic strains of *Escherichia coli* isolated from humans. Infect Immun 1982;**36**:189–97
60. Smyth CJ. Two mannose-resistant haemagglutinins on enterotoxigenic *Escherichia coli* of serotype O6:K15:H16 or H- isolated from travellers' and infantile diarrhoea. J Gen Microbiol 1982;**128**:2081–96
61. Smith HR, Scotland SM, Rowe B. Plasmids that code for production of colonization factor antigen II and en-

terotoxin production in strains of *Escherichia coli*. Infect Immun 1983;**40**:1236–9
62. Mullany P, Field AM, McConnell MM, Scotland SM, Smith HR, Rowe B. Expression of plasmids coding for colonization factor antigen II (CFA/II) and enterotoxin production in *Escherichia coli*. J Gen Microbiol 1983;**129**: 3591–3601
63. Smyth CJ. Serologically distinct fimbriae on enterotoxigenic *Escherichia coli* of serotype O6:K15:H16 or H-. FEMS Lett 1984;**21**:51–7
64. Levine MM, Ristaino P, Marley G, Smyth C, Knutton S, Boedeker E, Black R, Young C, Clements ML, Cheney C, Patnaik R. Coli surface antigens I and 3 of colonization factor antigen II-positive enterotoxigenic *Escherichia coli*: Morphology, purification, and immune responses in humans. Infect Immun 1984;**44**:409–20
65. Knutton S, Lloyd DR, Candy DCA, McNeish AS. Ultrastructural study of adhesion of enterotoxigenic *Escherichia coli* to erythrocytes and human intestinal epithelial cells. Infect Immun 1984;**44**:519–27
66. de Graaf FK, Roorda I. Production, purification, and characterization of the fimbrial adhesive antigen F41 isolated from calf enteropathogenic *Escherichia coli* strain B41M. Infect Immun 1982;**36**:751–8
67. Stirm S, Ørskov F, Ørskov I, Birch-Andersen A. Episome-carried surface antigen K88 of *Escherichia coli*. III. Morphology. J Bacteriol 1967;**93**:740–8
68. Thomas LV, Cravioto A, Scotland SM, Rowe B. New fimbrial antigenic type (E8775) that may represent a colonization factor in enterotoxigenic *Escherichia coli* in humans. Infect Immun 1982;**35**:1119–24
69. McConnell MM, Thomas LV, Day NP, Rowe B. Enzyme-linked immunosorbent assays for the detection of adhesion factor antigens of enterotoxigenic *Escherichia coli*. J Infect Dis 1985;**152**:1120–7
70. Tacket CO, Maneval DR, Levine MM. A new fimbrial putative colonization factor of enterotoxigenic *Escherichia coli* serotype O159:H4: purification, morphology and genetics. (Submitted for publication)
71. Deneke CF, Thorne GM, Gorback SL. Serotypes of attachment pili of enterotoxigenic *Escherichia coli* isolated from humans. Infect Immun 1981;**32**:1254–60
72. Honda T, Arita M, Miwatani T. Characterization of new hydrophobic pili of human enterotoxigenic *Escherichia coli*: a possible new colonization factor. Infect Immun 1984;**43**:959–65
73. Darfeuille-Michaud A, Forestier C, Joly B, Cluzel R. Identification of a nonfimbrial adhesive factor of an enterotoxigenic *Escherichia coli* strain. Infect Immun 1986; **52**:468–75
74. Changchawalit S, Echeverria P, Taylor DN, Leksomboon U, Tirapat C, Eampokalap B, Rowe B. Colonization factors associated with enterotoxigenic *Escherichia coli* isolated in Thailand. Infect Immun 1984;**45**:525–7
75. Gothefors L, Åhrén C, Stoll B. Barua DK, Ørskov F, Salek MA, Svennerholm A-M. Presence of colonization factor antigens on fresh isolates of fecal *Escherichia coli*: a prospective study. J Infect Dis 1985;**152**:1128–33
76. Evans DG, Graham DY, Evans DJ Jr, Opekun A. Administration of purified colonization factor antigens (CFA/I, CFA/II) of enterotoxigenic *Escherichia coli* to volunteers. Response to challenge with virulent enterotoxigenic *Escherichia coli*. Gastroenterology 1984;**87**:934–40
77. Levine MM, Morris JG, Losonsky G, Boedecker E, Rowe B. Fimbriae (pili) adhesins as vaccines. In: Lark D, Normark S, Brent-Uhlin E, eds. Molecular biology of microbial pathogenicity. London: Academic Press, 1986:143–5
78. Levine M, Black R, Clements ML, Morris JG, Losonsky G, Boedecker E, Rowe B. Fimbriae (pili) adhesins as vaccines. In: Abstracts of the 21st Joint Conference on Cholera. Bethesda, MD: US-Japan Cooperative Medical Science Program, 1985
80. Clements JD, El-Morshidy S. Construction of a potential live oral bivalent vaccine for typhoid fever and cholera-*Escherichia coli*-related diarrheas. Infect Immun 1984;**46**:564–9
81. Yamamoto T, Tamura Y, Yokota T. Enteroadhesion fimbriae and enterotoxin of *Escherichia coli*: genetic transfer to a streptomycin-resistant mutant of the *galE* oral-route live-vaccine *Salmonella typhi* Ty21a. Infect Immun 1985; **50**:925–8
82. DuPont HL, Formal SB, Hornick RB, Snyder MJ, Libonati JP, Sheahan DG, LaBrec EH, Kalas JP. Pathogenesis of *Escherichia coli* diarrhea. N Engl J Med 1971;**285**:1–9
83. Harris JR, Wachsmuth IK, Davis BR, Cohen ML. High-molecular-weight plasmid correlates with *Escherichia coli* enteroinvasiveness. Infect Immun 1982;**37**:1295–8
84. Hale TL, Sansonetti PJ, Schad PA, Austin S, Formal SB. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. Infect Immun 1983;**40**:340–50
85. Toledo MRF, Trabulsi LR. Correlation between biochemical and serological characteristics of *Escherichia coli* and results of the Serény test. J Clin Microbiol 1983;**17**:419–21
86. Pál T, Pácsa AS, Emödy L, Vörös S, Sélley E. Modified enzyme-linked immunosorbent assay for detecting enteroinvasive *Escherichia coli* and virulent *Shigella* strains. J Clin Microbiol 1985;**21**:415–8
87. Wood PK, Morris Jr JG, Small PLC, Sethabutr O, Toledo MRE, Trabulsi L, Kaper JB. Comparison of DNA probes with the Sereny test for identification of invasive *Shigella* and *Escherichia coli* strains. J Clin Microbiol 1986;**24**:498–500
88. Sack RB, Hirschhorn N, Brownlee I, Cash RA, Woodward WE, Sack DA. Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. N Engl J Med 1975;**292**:1041–5
89. Gross RJ, Scotland SM, Rowe B. Enterotoxin testing of *Escherichia coli* causing epidemic infantile enteritis in the U.K. Lancet 1976;**1**:629–31
90. Echeverria PD, Chang CP, Smith D, Anderson GL. Enterotoxigenicity and invasive capacity of "enteropathogenic" serotypes of *Escherichia coli*. J Pediatr 1976;**89**:8–10
91. Goldschmidt MC, DuPont HL. Enteropathogenic *Escherichia coli*: lack of correlation of serotype with pathogenicity. J Infect Dis 1976;**133**:153–6
92. Sack RB. Serotyping of *E. coli*. Lancet 1976;**1**:1132
93. Gurwith MJ, Wiseman DA, Chow P. Clinical and labora-

tory assessment of the pathogenicity of serotyped enteropathogenic *Escherichia coli*. J Infect Dis 1977;**135**: 736–43

94. Gangarosa EJ, Merson MH. Epidemiologic assessment of the relevance of the so-called enteropathogenic serogroups of *Escherichia coli* in diarrhea. N Engl J Med 1977; **296**:1210–3
95. Sack RB. Enterotoxigenic *Escherichia coli*—an emerging pathogen. N Engl J Med 1976;**295**:893–4
96. French GL. Enteropathogenic *E. coli*. Lancet 1976;**1**:1411–2
97. Levine MM, Bergquist EJ, Nalin DR, Waterman DH, Hornick RB, Young CR, Sotman S, Rowe B. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet 1978;**1**:1119–22
98. Polotsky YUE, Dragunskaya EM, Seliverstova VG, Avdeeva TA, Chakhutinskaya MG, Kétyi I, Vertényi A, Ralovich B, Emödy L, Málovics I, Safonova NV, Snigirevskaya ES, and Karyagina EI. Pathogenic effect of enterotoxigenic *Escherichia coli* and *Escherichia coli* causing infantile diarrhoea. Acta Microbiol Hung 1977;**24**:221–36
99. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol Rev 1984; **6**:31–51
100. Ulshen MH, and Rollo JL. Pathogenesis of *Escherichia coli* gastroenteritis in man—another mechanism. N Engl J Med 1980;**302**:99–101
101. Rothbaum R, McAdams AJ, Giannella R, and Partin JC. A clinicopathologic study of enterocyte-adherent *Escherichia coli:* a cause of protracted diarrhea in infants. Gastroenterology 1982;**83**:441–54
102. Tzipori S, Robins-Browne RM, Gonis G, Hayes J, Withers M, McCartney E. Enteropathogenic *Escherichia coli* enteritis: evaluation of the gnotobiotic piglet as a model of human infection. Gut 1985;**26**:570–8
103. Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Current Microbiology 1979;**3**:95–9
104. Baldini MM, Kaper JB, Levine MM, Candy DCA, and Moon HW. Plasmid-mediated adhesion in enteropathogenic *Escherichia coli*. J Pediatr Gastroenterol Nutr 1983; **2**:534–8
105. Scaletsky ICA, Silva MLM, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect Immun 1984;**45**:534–6
106. Nataro JP, Scaletsky ICA, Kaper JB, Levine MM, Trabulsi LR. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect Immun 1985;**48**:378–83
107. Levine MM, Nataro JP, Karch H, Baldini MM, Kaper JB, Black RE, Clements ML, O'Brien AD. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. J Infect Dis 1985;**152**:550–9
108. Nataro JP, Baldini MM, Kaper JB, Black RE, Bravo N, Levine MM. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J Infect Dis 1985;**152**:560–5
109. Marques LRM, Moore MA, Wells JG, Wachsmuth IK, O'Brien AD. Production of Shiga-like toxin by *Escherichia coli*. J Infect Dis 1986;**154**:338–41
110. Cleary TG, Mathewson JJ, Faris E, Pickering LK. Shiga-like cytotoxin production by enteropathogenic *Escherichia coli* serogroups. Infect Immun 1985;**47**:335–7
111. Toledo MRF, Alvariza MCB, Murahovschi J, Ramos SRTS, Trabulsi LR. Enteropathogenic *Escherichia coli* serotypes and endemic diarrhea in infants. Infect Immun 1983;**39**: 586–9
112. Prado V, Braun S, Bosch P, Bercovich M, Reyes L, Sawada M. Análisis de *Escherichia coli* enteropatógeno clásico (E.C.E.P) como causa endémica de diarrea aguda en niños Chilenos. Rev Chil Pediatr 1984;**55**:171–5
113. Black RE, Brown KH, Lopez de Ramona G, Lanata C, Becker S. Community-based studies of diarrhea in Bangladesh and Peru. In: Proceedings of the 34th Annual Meeting of the American Society of Tropical Medicine and Hygiene. Baltimore, 1985
114. Moseley SL, Clausen CR, Smith AL. A new bacterial adhesin associated with enteritis in infants. [abstract no. 1128]. In: Program and abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC: American Society for Microbiology, 1985
115. Cravioto AJ, Lince M, Carrillo J, Cancino G. Pathogenic ability of *Escherichia coli* strains and diarrheal disease in a cohort of rural infants. [abstract no. 47]. In: Program and abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC: American Society for Microbiology, 1986
116. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1982;**308**:681–5
117. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983;**1**:619–20
118. PHLS Communicable Disease Surveillance Centre. Haemolytic-uremic syndrome. Communicable Disease Report 1983;**36**:1
119. Centers for Disease Control. Hemolytic-uremic syndrome associated with *Escherichia coli* O157:H7 enteric infections—United States, 1984. MMWR 1985;**34**:20–1
120. Karmali MA, Petric M, Lim C, Fleming PC, Steele BT. *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis. Lancet 1983;**2**:1299–1300
121. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis 1985;**151**:775–82
122. Spika JS, Parsons JE, Nordenberg D, Wells JG, Gunn RA, Blake PA. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. J Pediatr 1986;**109**:287–91
123. Gransden WR, Damm MAS, Anderson JD, Carter JE, Lior H. Further evidence associating hemolytic uremic syndrome with infection by Verotoxin-producing *Escherichia coli* O157:H7. J Infect Dis 1986;**154**:522–4

124. Remis RS, MacDonald KL, Riley LW, Puhr ND, Wells JG, Davis BR, Blake PA, Cohen ML. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann Intern Med 1984;**101**:624–6
125. Pai CH, Gordon R, Sims HV, Bryan LE. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Clinical, epidemiologic, and bacteriologic features. Ann Intern Med 1984;**101**:738–42
126. Harris AA, Kaplan RL, Goodman LJ, Doyle M, Landau W, Segreti J, Levin S. Results from a four-month stool survey for *E. coli* O157:H7—use of a new screening method. Clin Res 1984;**32**:761A
127. Stewart PJ, Desormeaux W, Chene J. Hemorrhagic colitis in a home for the aged: Ontario. Can Dis Weekly Report 1983;**9**:29–32
128. Pai CH, Blore BG, Ahmed N, Sims HV, Woods DE. Epidemiology of diarrheal diseases due to *Escherichia coli* O157:H7 and other Verotoxin-producing *E. coli* [abstract no. C-574]. In: Proceedings of the annual meeting of the Canadian Society for Clinical Research, Vancouver, 1985
129. Johnson WM, Lior H, Bezanson GS. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. Lancet 1983;**1**:76
130. O'Brien AD, Lively TA, Chang TW, Gorbach SL. Purification of *Shigella dysenteriae* 1 (Shiga)-like toxin from *Escherichia coli* O157:H7 strain associated with haemorrhagic colitis. Lancet 1983;**2**:573
131. O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science 1984;**226**:694–6
132. Smith HR, Day NP, Scotland SM, Gross RJ, Rowe B. Phage-determined production of vero cytotoxin in strains of *Escherichia coli* serogroup O157. Lancet 1984;**2**:1242–3
133. Scotland SM, Smith HR, Rowe B. Two distinct toxins active on Vero cells from *Escherichia coli* O157. Lancet 1985;**2**:885–6
134. Karmali MA, Petric M, Louie S, Cheung R. Antigenic heterogeneity of *Escherichia coli* Verotoxins. Lancet 1986; **1**:164–5
135. Strockbine NA, Marques LRM, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986;**53**:135–40
136. Noda M, Nakabayashi N, Yutsudo T, Hirayama T, Takeda Y. Physico-chemical and biological properties of the purified Shiga-like toxin from *Escherichia coli* O157:H7. Toxicon 1985;**23**:600
137. Brown JE, Griffin DE, Rothman SW, Doctor BP. Purification and biological characterization of Shiga toxin from *Shigella dysenteriae* 1. Infect Immun 1982;**36**:996–1005
138. Eiklid K, Olsnes S. Animal toxicity of *Shigella dysenteriae* cytotoxin: evidence that the neurotoxic, enterotoxic, and cytotoxic activities are due to one toxin. J Immunol 1983;**130**:380–4
139. Karch H, Heeseman J, Laufs R, O'Brien AD, Tacket CO, Levine MM. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect Immun (in press)
140. Farmer JJ III, Potter ME, Riley LW, Barrett TJ, Blake PA, Bopp CA, Cohen ML, Kaufmann A, Morris GK, Remis RS, Thomason BM, Wells JG. Animal models to study *Escherichia coli* O157:H7 isolated from patients iwth haemorrhagic colitis. Lancet 1983;**1**:702–3
141. Beery JT, Doyle MP, Higley NA. Cytotoxic activity of *Escherichia coli* O157:H7 culture filtrate on the mouse colon and kidney. Current Microbiology 1984;**11**:335–42
142. Beery JT, Doyle MP, Schoeni JL. Colonization of chicken cecae by *Escherichia coli* associated with hemorrhagic colitis. Appl Environ Microbiol 1985;**49**:310–5
143. Potter ME, Kaufmann AF, Thomason BM, Blake PA, Farmer JJ III. Diarrhea due to *Escherichia coli* O157:H7 in the infant rabbit. J Infect Dis 1985;**152**:1341–3
144. Pai CH, Kelly JK, Meyers GL. Experimental infection of infant rabbits with Verotoxin-producing *Escherichia coli*. Infect Immun 1986;**51**:16–23
145. Tzipori S, Wachsmuth IK, Chapman C, Birner R, Brittingham J, Jackson C, Hogg J. The pathogenesis of hemorrhagic colitis caused by *Escherichia coli* O157:H7 in gnotobiotic piglets. J Infect Dis 1986;**154**:712–6
146. Francis DH, Collins JE, Duimstra JR. Infection of gnotobiotic pigs with an *Escherichia coli* O157:H7 strain associated with an outbreak of hemorrhagic colitis. Infect Immun 1986;**51**:953–6
147. O'Brien AD, LaVeck GD. Purification and characterization of *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect Immun 1983;**40**:675–83
148. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Lett 1980;**7**:15–7
149. Smith HW, Green P, Parsell Z. Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. J Gen Microbiol 1983;**129**:3121–37
150. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977;**18**:775–9
151. Wade WG, Thom BT, Evans N. Cytotoxic enteropathogenic *Escherichia coli*. Lancet 1979;**2**:1235–6
152. McNeish AS, Turner P, Fleming J, Evans N. Mucosal adherence of human enteropathogenic *Escherichia coli*. Lancet 1975;**2**:946–8
153. Smith HW, Linggood MA. The transmissible nature of enterotoxin production in a human enteropathogenic strain of *Escherichia coli*. J Med Microbiol 1971;**4**:301–5
154. Williams PH, Sedgwick MI, Evans N, Turner PJ, George RH, McNeish AS. Adherence of an enteropathogenic strain of *Escherichia coli* to human intestinal mucosa is mediated by a colicinogenic conjugative plasmid. Infect Immun 1978;**22**:393–402