INFECTION AND IMMUNITY, Apr. 1989, p. 1142–1150
0019-9567/89/041142-09$02.00/0

Vol. 57, No. 4

Nature and Distribution of Mucosal Lesions Associated with Enteropathogenic and Enterohemorrhagic *Escherichia coli* in Piglets and the Role of Plasmid-Mediated Factors

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Received 18 July 1988/Accepted 17 December 1988

Bacterial attachment-effacement (att-eff) is emerging as an important virulence characteristic common to both enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). The contribution of the plasmid-encoded EPEC adherence factor to the production of mucosal lesions and diarrhea was investigated in gnotobiotic piglets. Bacterial att-aff in the intestinal mucosa of piglets infected with plasmid-cured EPEC strain E2348/69 (O127) was indistinguishable from that in piglets infected with the parent strain, but the distribution of lesions was different; it occurred in the small intestines of 6 of 7 piglets infected with the parent strain compared with only 2 of 11 ($P = 0.006$) infected with the plasmid-cured strain. Plasmid-encoded factors in EPEC and EHEC strains did not appear to contribute to bacterial competition with normal gut microflora. Of 13 strains belonging to five EPEC serogroups, O55, O142, O26, O119, and O111, 3 fulfilled the criteria for EHEC (2 O26 and 1 O111). There were three distinct patterns of bacterial association with the intestinal mucosa of infected piglets. (i) EHEC strains caused bacterial att-eff associated with extensive destruction of surface and glandular epithelia in the large intestines with little or no inflammatory response. (ii) Some EPEC strains caused severe diarrhea which correlated with the extent of bacterial att-eff in the proximal small intestine, disruption of the epithelial cell membrane, and inflammation. It is suggested that, with respect to virulent strains, this degree of involvement determines the clinical outcome. Mildly pathogenic strains (O127 and O119), in which bacterial att-eff was restricted to the distal halves of the small and large intestines, caused little or no diarrhea. In such strains, nonimmune host factors (smaller, poorly feeding, and lethargic piglets) tended to play a determining role with regard to the degree of involvement of the small intestine and hence the clinical outcome. (iii) One strain (O55) caused illness and mucosal damage which could not be accounted for by the sparse bacterial att-eff observed in the gut. Instead, bacteria penetrated into and proliferated in the lamina propria, undermining the villous tips in the small intestine. Bacterial att-eff was the most important virulence factor in most of the strains examined, but plasmid-mediated factors facilitated bacterial adhesion in the small intestine, which may explain the reduced pathogenicity of the plasmid-cured variant of strain E2348/69 for human volunteers.

Enteropathogenic *Escherichia coli* (EPEC) strains are considered a homogeneous group, distinct from other diarrheagenic *E. coli* strains—namely, enterotoxigenic *E. coli*, enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* strains—chiefly because they lack specific virulence attributes which are characteristic of the others (19). Until recently, the evidence for the enteropathogenicity of EPEC strains was based on epidemiological and clinical observations, and serogrouping was the method used for their identification.

The characteristic way in which all EPEC strains associate with the intestinal mucosa, as observed in human intestinal biopsies (3, 20, 22, 28) and experimental animals (15, 21, 25), is known as attachment-effacement (att-eff) (15). In the region of bacterial attachment to enterocytes, the microvillous border is lost, the cytoskeletal elements are disrupted, and there is marked modification and eventual disruption of the epithelial cell membrane. These cellular changes, the mucosal response to them and to the adhering bacteria—namely, inflammation—and their effects on the physiology of digestion and absorption are presumably the key to the pathogenesis of the disease.

There appear to be two genetically independent components which facilitate bacterial association with the cell surface which have been demonstrated only in vitro (8, 9), viz., (i) the EPEC adherence factor (EAF), a plasmid-mediated characteristic highly conserved among EPEC strains (17), which is demonstrated in HEp-2 cells (3, 4), and (ii) att-eff by bacteria, which induces cellular alterations similar to those observed in human mucosal biopsies and experimental animals (3, 15, 21, 25, 28). These in vitro findings need to be confirmed in vivo. Studies in human volunteers have shown that EAF is required for full expression of EPEC enteropathogenicity (11), but the relative contributions of EAF and att-eff to establishment and proliferation of bacteria in the lumen, penetration of mucous gel, and attachment to surface cells are unknown.

In contrast, EHEC strains have three putative virulence attributes, viz., production of one or more Shiga-like toxins (SLT; 18) and plasmid-mediated fimbriae (6), both of which are demonstrable in cultured cells, and att-eff, which is demonstrable in the large intestines of piglets (5, 26). Unlike EPEC, EHEC induces a minimal inflammatory response (24, 27).

The aims of this study were (i) to determine whether plasmid-encoded factors of EPEC and EHEC influence colonization in the gastrointestinal (GI) tract or the extent and location of bacterial att-eff and (ii) to study the nature and distribution of mucosal lesions in the GI tracts of piglets

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TABLE 1. Sources and characteristics of the *E. coli* strains used in this study

Strain	Serogroup	Plasmid profile[a]	SLT product	Obtained from:[b]	Reference
E2348/69	O127	EP		CVD	10
E2348/69c	O127			CVD	1
933	O157	EH	I, II	CVD	23
933c	O157		I, II	CVD	23
55/MDU	O55	EP		MDU	
28-2	O55	EP		CVD	
E851/71	O142	EP		CVD	10
E2381/70	O142	EP		CVD	24
H311B	O26			IMVS	
26/MDU	O26	EP		MDU	
EH6/RCH	O26	EH	II	RCH	
H30	O26	EH	I	CDC	17
18-1	O119	EP		MDU	
119/MDU	O119			MDU	
ST/W	O111	EP		IMVS	
13-3	O111	EP		CVD	
3007-85	O111	EH	I, ?	CDC	25
K-12 (600)				CDC	23

[a] Presence of a 55- to 60-megadalton plasmid which hybridized with either an EPEC (EP) or an EHEC (EH) DNA probe.

[b] CVD, Center for Vaccine Development, Baltimore, Md.; CDC, Centers for Disease Control, Atlanta, Ga.; MDU, Microbiology Diagnostic Unit, University of Melbourne, Melbourne, Australia; IMVS, Institute of Medical and Veterinary Sciences, Adelaide, Australia; RCH, Royal Children's Hospital, Melbourne, Australia.

to highlight distinguishing features among EPEC and EHEC strains.

MATERIALS AND METHODS

Bacteria. The bacterial strains used in this study are listed in Table 1. EPEC strain E2348/69 and its plasmid-cured variant and E851/71 and E2381/70 (1, 10, 11) and EHEC strain 933 and its plasmid-cured variant and H30 and 3007-85 (18, 24, 27) have been studied previously.

Preparation of inocula. Bacteria were stored at −70°C in 34% (vol/vol) glycerol. Before use, they were cultured on 5% horse blood agar at 37°C overnight. About 20 colonies of each strain were inoculated into 50 ml of tryptic soy broth containing 0.6% (wt/vol) yeast extract. Flasks were incubated at 37°C overnight with shaking. Bacteria were pelleted by centrifugation and resuspended in 1/10 of the original volume of spent medium. The number of bacteria inoculated was adjusted to contain about 10^{10} CFU given in 1 ml, except for strain 3007-85, for which an inoculum of 10^8 was used because of its high virulence for piglets (27). The bacteria used to inoculate piglets were characterized by serotyping, biotyping, and plasmid profile (24). The plasmid content of each strain was determined by performing agarose gel electrophoresis on DNA extracted from bacteria (2). Strains were classified as EPEC or EHEC by hybridization reaction with the corresponding DNA probe (12, 16) (probes were kindly provided by the Center for Vaccine Development, University of Maryland, Baltimore). The multiple typing scheme was also used to confirm the identities of bacteria recovered from the intestinal contents or heart blood of infected piglets.

Experimental animals. Either gnotobiotic (GB) (13) or conventional (CN) healthy newborn piglets that had received colostrum for 24 h and had acquired gut microflora were used for these experiments. CN piglets were placed into plastic microbiological isolators within 24 h of birth. The piglets were inoculated orally and housed in pairs. Each bacterial strain was tested at least twice in animals from separate litters. The piglets were observed regularly for signs of diarrhea and illness. They were usually inoculated within 24 h of birth and were euthanized by injection of sodium pentobarbitone at 1 to 5 days after inoculation or when severe illness developed.

TABLE 2. Effects of plasmid-encoded factors on extent of bacterial colonization of the small and large intestines of newborn piglets and the influence of normal microflora

E. coli strain[a]	No. of piglets	No. with diarrhea[b]	No. with gut colonization	No. with bacterial att-eff	
				Small intestine	Large intestine
E2348/69	8 (GB)[c]	3	8	6	7[d]
E2348/69c	12 (GB)[c]	2	12	2	11[d]
E2348/69	6	0	4	2	3
E2348/69c	6	0	4	0	3
933	8	3	5	0	5
933c	10	3	6	0	6

[a] Strain E2348/69 is EPEC serotype O127:H6, and E2348/69c is its plasmid-cured variant; strain 933c is a plasmid-cured variant of EHEC strain 933, serotype O157:H7.

[b] The animals were killed 1 to 2 days after oral inoculation with strain E2348/69 and 2 to 3 days after inoculation with strain 933.

[c] GB piglets; the remaining animals were CN piglets with normal gut microflora.

[d] No lesions were seen in one piglet killed 5 days after inoculation.

Necropsy procedure. The piglets were examined for gross pathological changes. Samples were taken from five equally spaced sites in the small intestine and from the cecum, colon, and mesenteric lymph nodes. After fixation in buffered Formalin, samples were sectioned for light and electron microscopy (26). The proximal, mid, and distal small intestine; colon; and heart blood were cultured quantitatively (26).

Cytotoxicity assay. All of the strains were assayed for cytotoxicity for SLT-sensitive HeLa cells (kindly supplied by I. K. Wachsmuth, Centers for Disease Control, Atlanta, Ga.) as described previously (24). Culture fluids from strains cytotoxic for HeLa cells were further tested in a neutralization assay with rabbit SLT-I and SLT-II antisera.

Roles of plasmid-mediated factors. Twenty GB and 32 CN piglets were orally inoculated with 10^{10} CFU of EPEC strain E2348/69 (O127:H6), EHEC strain 933 (O157:H7), or the respective plasmid-cured derivative E2348/69c or 933c. Control animals were inoculated with *E. coli* C600 (K-12). The number of piglets used for each strain is detailed in Table 2.

Nature and distribution of mucosal lesions. Groups of at least four piglets were each orally inoculated with 1 of 14 *E. coli* strains (listed in Table 3) belonging to five different classical (O142, O26, O119, O111, and O55) EPEC serogroups. Three strains belonging to serogroups O111 and O26 have been reclassified as EHEC strains on the basis of DNA hybridization reaction and production of SLT.

RESULTS

Roles of plasmid-mediated factors. The roles of plasmid-mediated factors in bacterial colonization are summarized in Table 2. Only 5 of 20 GB and none of 12 CN piglets inoculated with EPEC strain E2348/69 or its variant developed diarrhea. All 20 GB piglets were heavily colonized (range, 10^6 to 10^{11} bacteria per g of mucosal scrapings), and

TABLE 3. Distribution of att-eff by bacteria in the gut mucosa of newborn GB piglets orally inoculated with 1 of 13 *E. coli* strains

E. coli serogroup and strain	No. of piglets	No. with diarrhea (severity)[a]	No. with bacterial att-eff		
			Small intestine		Large intestine
			Proximal	Distal	
O142					
E851/71	4	4 (S)	3	4	4
E2381/70	4	4 (S)	2	4	4
O26					
H311B	4	4 (M)		3	4
26/MDU	4			1	3
EH6/RCH[b]	4	4 (S)		1	4
H30[b]	8				
O119					
18-1	4	3 (L)	3	4	4
199/MDU	4			2	2
O111					
ST/W	4	4 (L)	2	4	4
13-3	4	1 (M)		2	4
3007-85[b]	4	4 (S)		1	4
O55					
55/MDU	8	8[c]		2	5
28-2	4	4 (S)	3	4	4
K-12 (600)	2				

[a] S, Severe diarrhea; M, moderate diarrhea; L, light or mild diarrhea.
[b] Strains produce SLT and hybridize with a EHEC DNA probe (see text). Strain 3007-85, which induced considerably more-severe illness, was studied in more detail elsewhere (27).
[c] Acute illness (depression, anorexia, and bacteremia), often without diarrhea.

those killed within 2 days had mucosal lesions of various degrees of severity, with characteristic bacterial att-eff in the large intestines. Six of 7 GB piglets inoculated with E2348/69 but only 2 of 11 inoculated with its plasmid-cured variant ($P = 0.006$; Fisher's exact test) had bacterial att-eff of various degrees in the small intestines. No lesions were seen in one animal from each group killed 5 days after inoculation (Table 2).

A similar trend was observed in CN piglets. Strains E2348/69 and E2348/69c equally colonized the GI tracts (range, 10^3 to 10^8) of four of six CN piglets in each groups, and bacterial att-eff was observed in the large intestines of three of the four colonized piglets in each group. However, bacterial att-eff in the distal small intestine was seen only in piglets inoculated with E2348/69 (two of three).

Of 18 CN piglets, 6 inoculated with EHEC strains 933 and 933c had diarrhea, 11 were colonized, and all had characteristic bacterial att-eff in the large intestines (Table 2). GB piglets are consistently colonized by both strains (24).

Nature and distribution of lesions. Thirteen strains representing five *E. coli* serogroups induced a spectrum of clinical manifestations and variable distribution and severity of mucosal lesions. Three strains were EHEC, and the remaining 10 were EPEC, including 2 unconfirmed strains that lacked the appropriate plasmid (H311B and 119/MDU). Strains 26/MDU, H30 (O26), 199/MDU (O119), and 13-3 (O111) were either nondiarrheagenic or only mildly diarrheagenic for GB piglets.

Profuse diarrhea within 20 h, caused by mucosal lesions associated with extensive bacterial att-eff and marked leukocyte infiltration throughout most of the GI tract, was observed in animals inoculated with EPEC strains E851/71, E2381/70 (both O142), and 28-2 (O55). Strains 18-1 (O119) and ST/W (O111) were slightly less virulent.

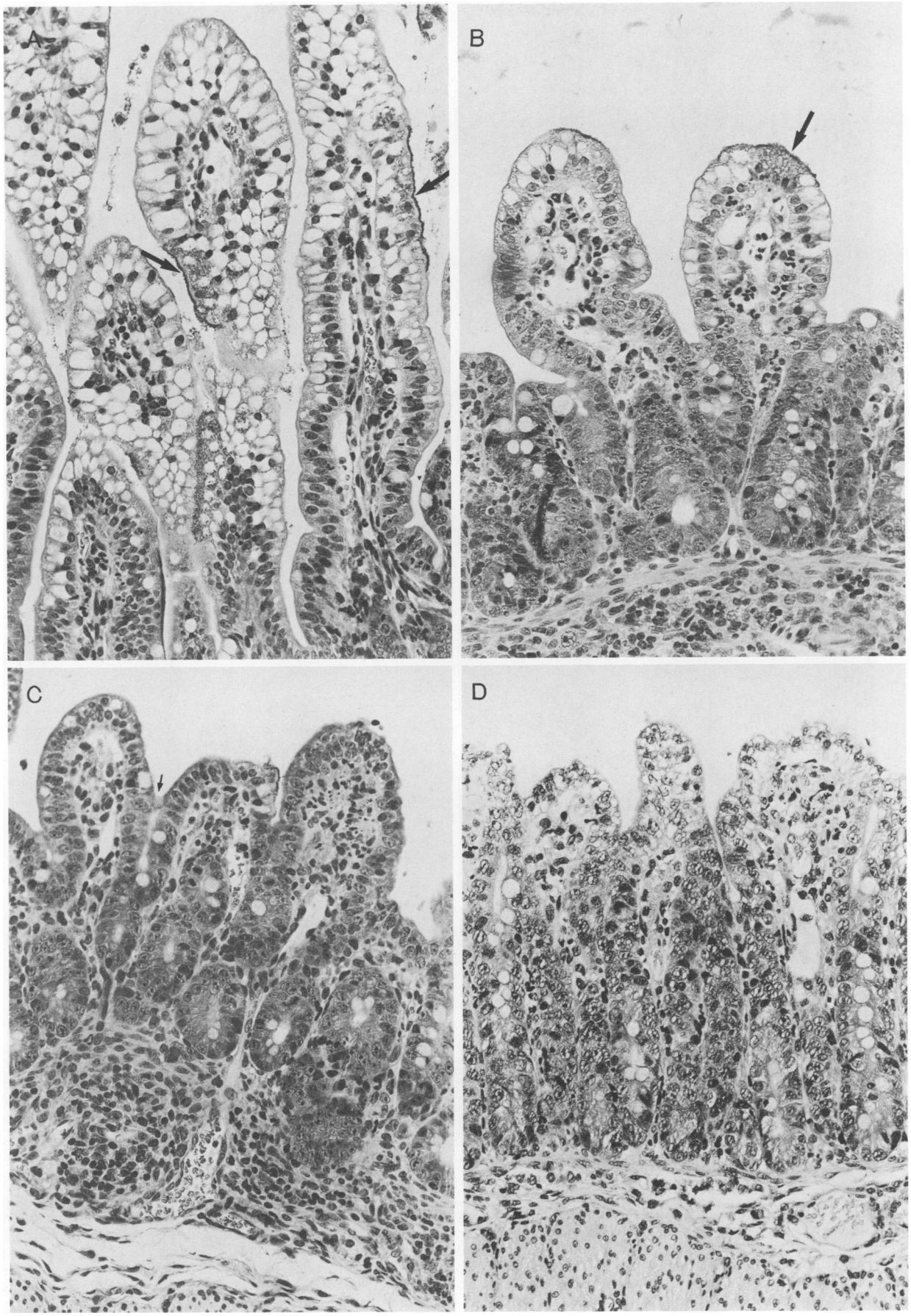
Inoculation of four piglets with strain 28-2 is described in some detail. Two piglets from the same litter, housed together and inoculated at the same time, developed diarrhea which was severe and acute in one and mild in the other. The severity correlated with the extent and severity of mucosal injury associated with bacterial att-eff. In piglet 1, it extended from the duodenum, involving 75% of the villi in the jejunum, compared with fewer than 5% in the jejunum in piglet 2. Changes were still more extensive in the ileum of the first piglet but were similar in the large intestines of the two piglets. The reason for the difference is not clear, since both animals were otherwise similar.

When the mucosa of a similarly inoculated piglet was examined 72 h after inoculation, the mucosa of the large intestine and part of the distal small intestine was flat, hyperemic, and extensively infiltrated with inflammatory cells. The surface epithelium was irregular, eroded in some areas, and depleted of goblet cells, and most of the surviving cells were vacuolated (Fig. 1). The appearance of extensive villous atrophy with fusion and elongation of crypts in the distal small intestine was reminiscent of mucosal changes seen in experimental viral infection (23). Five days after inoculation, the surviving fourth piglet still had diarrhea. There were very few foci of bacterial att-eff in the terminal ileum and cecum, but the mucosa, although showing signs of recovery, was still extensively damaged.

The four strains of serogroup O26 differed markedly from one another. EH6/RCH was a typical EHEC (it hybridized with an EHEC DNA probe, liberated SLT-II, and induced EHEC-like mucosal lesions [Fig. 2 and 3] which caused diarrhea in 3 to 4 days). H311B, which possessed no 55- to 60-megadalton plasmid and did not liberate SLT, induced diarrhea within 3 to 4 days and produced EHEC-like mucosal lesions. H30 hybridized with an EHEC DNA probe and produced extremely high levels of SLT-I but did not cause diarrhea, mucosal changes, or bacterial att-eff in the gut. Strain 26/MDU hybridized with an EPEC DNA probe, induced extremely sparse lesions, and caused no diarrhea.

The three strains of serogroup O111 also behaved differently from one another. Two were EPEC strains (ST/W and 13-3), but only the former induced lesions in the small intestine and diarrhea (Table 3). The third (3007-85) was a typical EHEC strain (27) but caused much more severe

FIG. 1. Histological sections of small intestines which illustrate successive mucosal changes seen in piglets necropsied at 1 (A and B), 2 (C), and 3 (D) days after inoculation with EPEC strain 28-2 (O55) (hematoxylin plus eosin stain; magnification, ×91). (A) lower jejunum showing normal-length villi. Note the extensive bacterial attachment to the surface epithelium and the deeper invasion of necrotic cells (arrows) by the bacteria. Vacuolation at this site in piglets is normal. (B) Terminal ileum showing swollen, shortened, and infiltrated villi, with bacteria adhering to and invading dying epithelial cells (arrow) at the tips. (C) Same site as that shown in panel B (slightly oblique) at 1 day later. Note the further reduction of villous height, with evidence of fusion (arrow). The surface is irregular, and the lamina propria is dense and contains many pyknotic cells. (D) Same site as that shown in panel B at 2 days later. Note the stunted and fused villi with irregular and poorly defined surfaces and elongated crypts; cell infiltration has subsided.


A
B
C
D

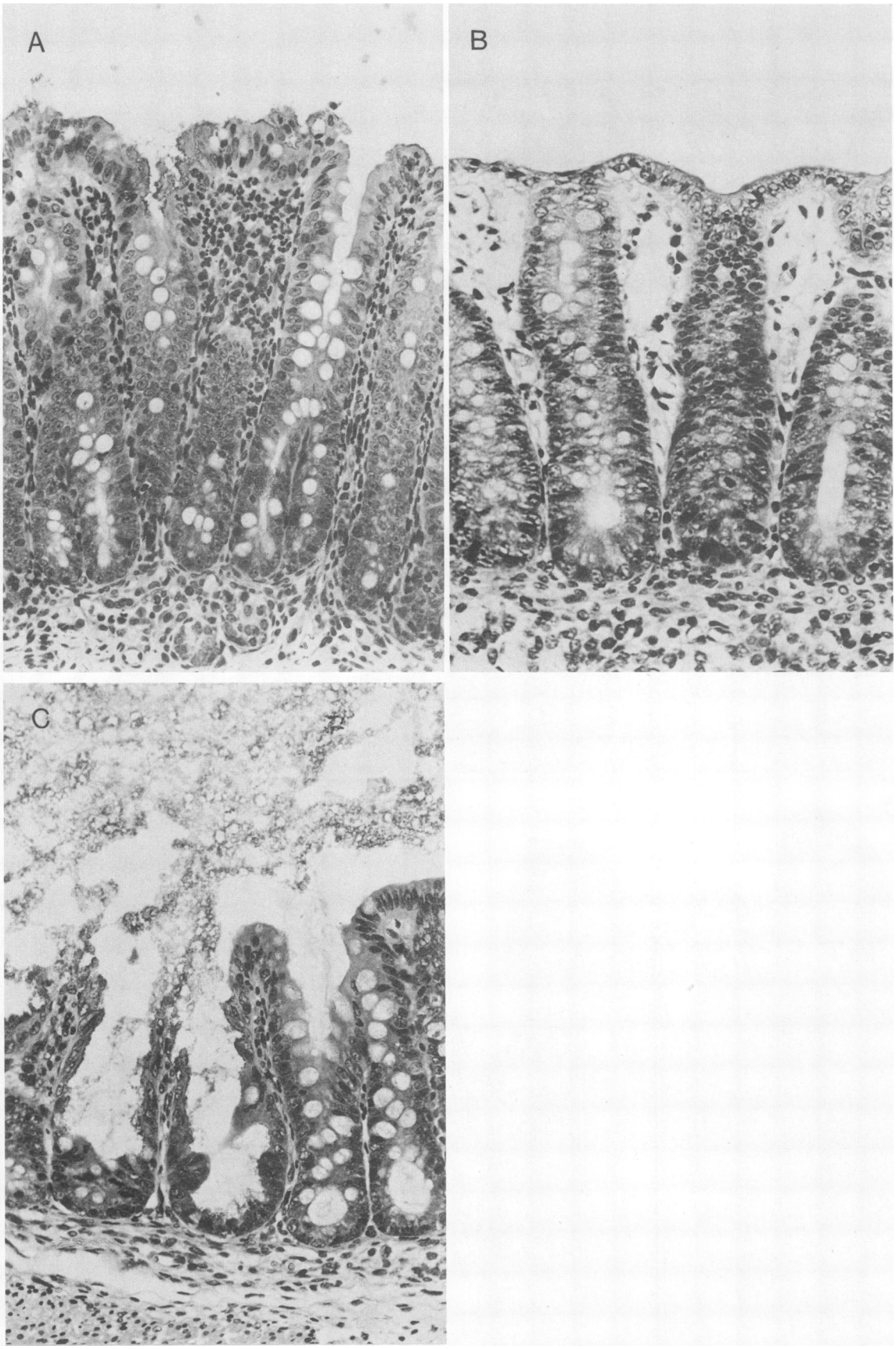


FIG. 2. Histological sections illustrating the contrast between early (A) and late (B) infections of the cecum with EPEC and infection with EHEC (C) (hematoxylin plus eosin stain; magnification, ×100). (A) One day after inoculation with strain 28-2. Note the bacterial att-eff throughout the surface epithelium, heavy cellular infiltration into the lamina propria, and intact crypts. (B) Same site as that shown in panel A at 3 days after inoculation with strain 28-2. Note that the surface epithelium is flat, irregular, vacuolated, and depleted of goblet cells; cell infiltration has subsided. (C) Same site as that shown in panel A at 3 days after inoculation with strain 6EH/RCH. Note the extensively damaged surface and glandular epithelia without cellular infiltration. Unaffected, intact mucosa is on the left.

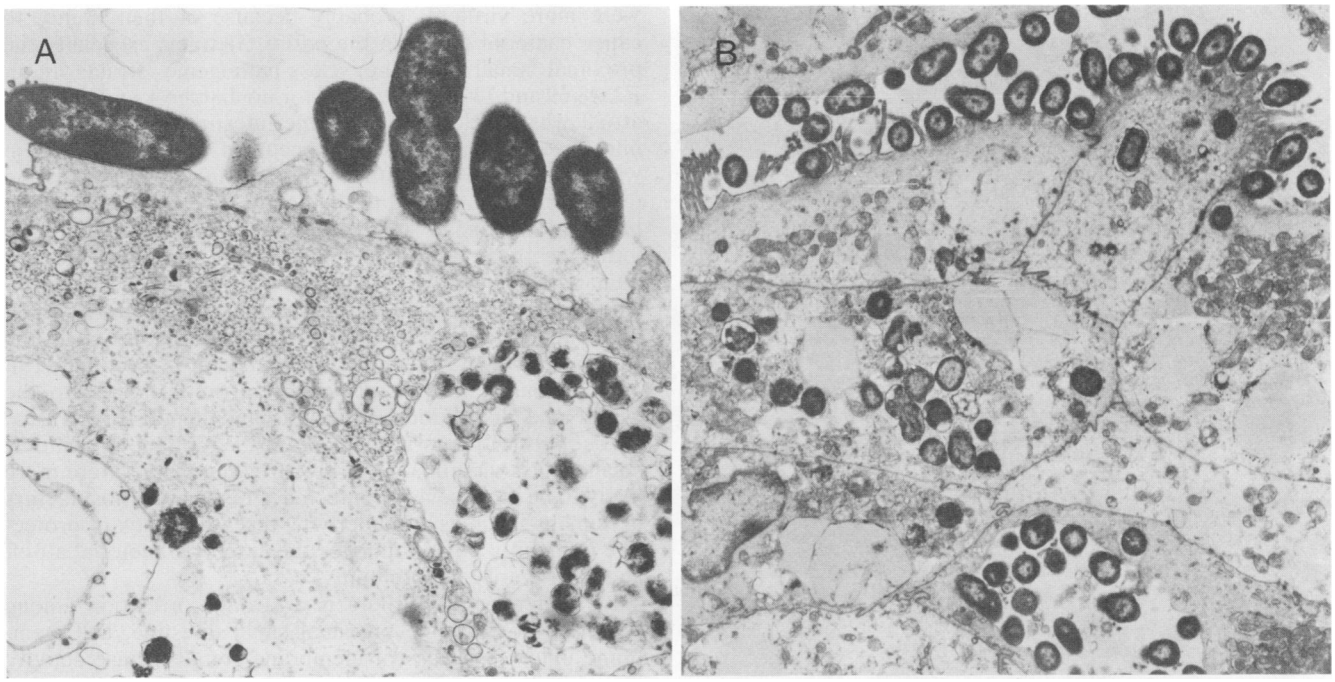


FIG. 3. Electron micrographs of ultrathin sections showing bacterial att-eff and modification of the cell membrane caused by EPEC (A) and EHEC (B). Note the remnants of bacteria within the cytoplasm and vesicles of a live epithelial cell in the mid-ileum of a piglet infected with EPEC strain 2348/69 (magnification, ×15,000). In contrast, the bacteria appear to be morphologically intact inside epithelial cells in the cecum of a piglet infected with EHEC strain EH6/RCH (magnification, ×3,000).

disease in the piglets, with systemic involvement, than any other EHEC or EPEC strain studied in GB piglets.

Eight GB piglets inoculated with strain 55/MDU (O55) suffered mild-to-moderate diarrhea, complicated by depression, anorexia, and coma in some animals. Although several foci of bacterial att-eff were seen in the terminal ilea and large intestines of half of the animals (Table 3), they were too few to account for either the profound clinical manifestations observed or the mucosal changes. Throughout the small intestines, bacteria attached to the microvillous border and penetrated the mucosa between enterocytes into the lamina propria (and presumably through capillaries into the blood stream), in which they proliferated, undermining the villous tips (Fig. 4). Six of eight piglets were bacteremic. These mucosal changes were not characteristic of EPEC or other diarrheagenic *E. coli* strain. The large intestines of these animals appeared unaffected.

Control strain c600, a derivative of K-12, caused no diarrhea or mucosal lesions in the two inoculated piglets, despite a degree of proliferation in the gut which was only marginally less (10^5 to 10^9) than that of pathogenic strains.

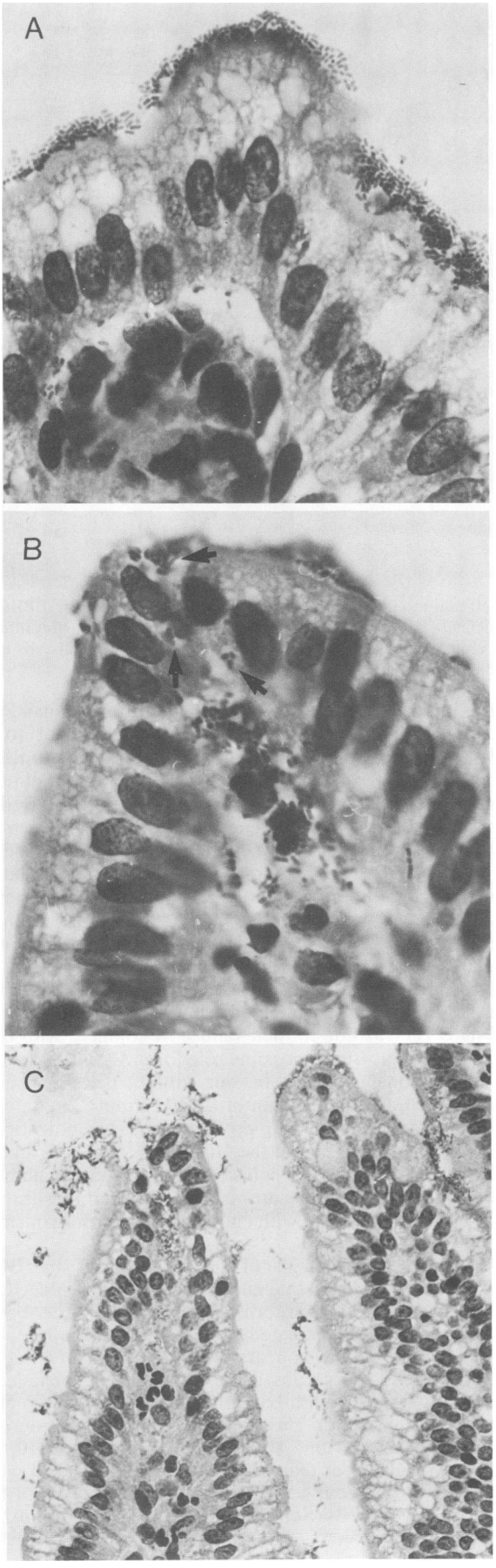
The responses of individual piglets, even those from the same litter, to specific strains often varied in the extent and distribution of lesions and the severity of clinical illness. Generally, piglets that were smaller, slow drinkers, and lethargic were more likely to develop illness with shorter incubation periods and become comatose and bacteremic than were their more active counterparts. They had higher bacterial counts (10 to 100 times higher) in the GI tract, more extensive and widely distributed bacterial att-eff, and more-reactive mucosa. The differences were most apparent with mildly pathogenic strains.

DISCUSSION

No definitive pathogenic role was established for the 55- to 60-megadalton plasmid (pMAR-2) of EPEC strain E2348/69 in this model, but clearly it is not directly responsible for bacterial att-eff of enterocytes. The same applies to the EHEC plasmid in the same animal model (24) and to that of strain E2348/69 in vitro with HEp-2 cells and cells cultured from duodenal biopsies (8, 9). Plasmid-mediated EAF (demonstrated as localized adherence in HEp-2 cells) appears to facilitate bacterial att-eff (8), presumably by increased adherence to cells. Our findings support this; bacterial att-eff was observed more frequently in the small intestines of piglets infected with strain E2348/69 than in those of piglets infected with its plasmid-cured variant, although there were similar bacterial counts in the GI tracts of both groups. We had assumed that the absence of bacterial att-eff in colostrum-deprived piglets infected with plasmid-cured strain E2348/69 (1) was due to their failure to compete with other microflora, indicating a possible role for pMAR-2. However, in conventional animals in our study, neither plasmid pMAR-2 of E2348/69 nor that of EHEC strain 933 conferred any advantage in promoting proliferation or colonization of the corresponding strain in the GI tract. Not all CN animals became colonized with *E. coli* strains pathogenic to humans, despite the high infectious dose, suggesting that these strains are less able to compete with endogenous microorganisms in the GI tracts of piglets.

Although establishment of bacterial att-eff by EPEC strains in the small intestine may be facilitated by pMAR2, in EHEC strains which are restricted to the large bowel, removal of plasmid-mediated factors, including one which codes for fimbriae (6), appears to be less crucial (24).

Of the 18 *E. coli* strains listed in Table 1, 7 behaved as truly EPEC strains, with bacterial att-eff and inflammatory cell infiltration. With classical EPEC strain (serogroups O127 and O142 and one strain each from O55, O119, and O111), a spectrum of diarrheal illness was observed which correlated strongly with the extent and distribution of bacterial att-eff (Table 3). Some strains (e.g., E851/71 and 28-2)



were more virulent, probably because of their ability to cause bacterial att-eff in the entire GI tract, especially the proximal small intestine. Less-pathogenic strains (e.g., E2348/69 and 13-3) caused little or no diarrhea, and bacterial att-eff was restricted to the large intestine and distal small intestine. Diarrhea generally occurred only in animals in which more than half of the small intestine was affected, and the severity was related to the degree of involvement of the proximal region. It is tempting to attribute the greater virulence of strain E851/71 than strain E2348/69 (10) for human volunteers to a mechanism identical to that in piglets. As for E2348/69, the lack of a plasmid in strain 199/MDU may explain its lesser production of bacterial att-eff in the small intestine and diarrhea compared with 18-1 (both O119).

Nonimmune host factors apparently contributed to the extent and distribution of lesions and hence to diarrhea, particularly with less-virulent strains. Within the same litter, smaller, less-vigorous, and slow-drinking piglets were more likely to be more severely affected. Presumably, host factors in humans, such as an underlying disease or lack of protective immunity, could influence the extent of bacterial att-eff and the severity of symptoms.

In virulent strains, there is a strong correlation among bacterial att-eff in the proximal small intestine, cell membrane injury, and loss of membrane-bound lactase activity (25). Similarly, enzyme activity was reduced in a human jejunal biopsy in which bacterial att-eff due to EPEC O111 was present (22). Acute diarrhea due to virulent strains (e.g., E851/71) presumably results from maldigestion and malabsorption due to direct effects on different parts of the small and large intestines. Attachment of bacteria in the proximal small intestine is much more difficult than in the distal half because of physiological barriers associated with extreme pH, high flow rate, and proteolytic activity. In contrast, diarrhea due to EHEC strains develops after 3 to 4 days as a result of reduced absorption and loss of fluid, electrolytes, and plasma proteins secondary to severe mucosal damage in the large bowel.

A comparison between E851/71 and its plasmid-cured variant, instead of E2348/69, in piglets might have helped elucidate the role of EAF and other plasmid-mediated factors, but attempts to cure this strain have been unsuccessful.

These results support the view that bacterial att-eff is the most important virulence factor of EPEC and is chromosomally encoded. However, the factors that determine the degree and site of attachment (including the difference between EPEC and EHEC strains), and hence the severity of disease, remain unknown. Our study and one with human volunteers (11) suggest that EAF is not an essential virulence factor, although it may be required for full expression of pathogenicity.

Of four strains which belonged to serogroup O26 and had various combinations of putative virulence factors, three induced EHEC-like mucosal lesions, but only two caused

FIG. 4. Histological sections from the terminal ileum of a piglet inoculated with EPEC strain 55/MDU (O55), showing stages of attachment, penetration, and proliferation of bacteria inside the villous lamina propria (hematoxylin plus eosin). (A) Bacteria adhering (not by EPEC att-eff) to surface epithelium; several organisms have penetrated the lamina propria (magnification, ×340). (B) Large numbers of bacteria in the lamina propria and in small groups between epithelial cells (arrows) can be seen undermining the villous tip (magnification, ×410). (C) The villous tip is disrupted by an outpouring of bacteria and, presumably, various tissue constituents (magnification, ×150).

diarrhea within 3 to 4 days which was unrelated to the type or presence of a plasmid and liberation of SLT.

Bacterial att-eff caused by EHEC strains has not yet been shown to be an important virulence factor in the large bowel in humans (7), since it would require biopsies to be taken from the ileocecal region during acute infection.

Some strains of both EHEC and EPEC possess additional virulence characteristics. For instance, with strain 55/MDU (O55), which did induce bacterial att-eff, mucosal lesions were largely attributed to bacterial proliferation in the lamina propria, leading to disruption of villous tips. Strain 3007-85 (O111) induced a much more acute illness, with systemic involvement, unlike other EHEC strains (27). Similar effects were produced by a plasmid-cured variant (data not shown).

In investigating the nature of the mucosal injury attributed to infections with human EPEC and EHEC strains in the piglet model (24–27), we recognized three distinct patterns with variations, suggesting that certain strains have additional unidentified virulence factors. (i) With EPEC strains, the degree of mucosal injury depended on the extent of bacterial att-eff of the surface epithelium and the intensity of the inflammatory reaction, especially in the small intestine. (ii) EPEC strain 55/MDU induced mucosal injury which was distinct from that caused by other diarrheagenic *E. coli* strains; bacteria penetrated and proliferated within the lamina propria, which undermined the villous tips. (iii) With EHEC strains, bacterial att-eff was restricted to the large intestine, and the resulting damage, involving the surface and glandular epithelia, was much more extensive, but there was little or no inflammatory reaction. SLT has no direct effect on the mucosa in newborn piglets (24) (as shown with strain H30), but it may indirectly affect the vasculature of the large intestine. It is postulated that SLT is absorbed from the injured mucosa and acts systemically by inducing injury to the endothelia of small blood vessels and capillaries in selected sites. In piglets, this includes the colon, where it causes increased permeability (edema) and congestion, producing a jellylike appearance. This was not seen in piglets infected with highly SLT-producing EHEC strain H30, in which the mucosa remained intact, in contrast to those infected with EHEC strains that induce mucosal injury. However, if the SLT microvillus-binding receptors in pigs appear at an older age, as in rabbits (14), SLT could, in addition, act directly on the mucosa.

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