INFECTION AND IMMUNITY, Apr. 1991, p. 1507–1513
0019-9567/91/041507-07$02.00/0

Vol. 59, No. 4

# *Hafnia alvei*, a Probable Cause of Diarrhea in Humans

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Received 20 August 1990/Accepted 25 January 1991

***Hafnia alvei*, a member of the family *Enterobacteriaceae*, was the only species of bacteria cultured from the stool of a 9-month-old child who was admitted with a 3-day history of watery diarrhea. The isolated strain of *H. alvei* failed to produce heat-labile or heat-stable enterotoxins or Shiga-like toxin I or II and did not invade HeLa cells, nor did it cause keratoconjunctivitis (determined by the Sereny test) in a guinea pig's eye. The strain, however, induced diarrhea in 8 of 12 adult rabbits with removable intestinal ties (removable intestinal tie-adult rabbit diarrhea [RITARD] assay) and in 1 of 2 orally fed animals. No diarrhea could be induced with *Escherichia coli* K-12 in eight RITARD assay rabbits and three orally fed rabbits, respectively. Microscopic examination of affected animals revealed moderate inflammatory cellular infiltration of the intestinal mucosa, in which bacterial attachment to the surface epithelium and loss of the microvillus border were evident in the ileum and colon. Electron microscopy demonstrated cellular modifications of the apical surface, with cupping or pedestal formation and increased terminal web density at sites of bacterial "attachment-effacement," a well-known characteristic and mechanism of diarrhea of enteropathogenic *E. coli*. Identical lesions were also induced by *H. alvei* in rabbit ileal loops, which ruled out naturally occurring rabbit enteropathogenic *E. coli* strains, which are known to produce similar lesions. It is concluded that at least some strains of *H. alvei* have the potential to cause diarrhea and that attachment-effacement is a virulence characteristic shared by bacteria other than *E. coli*.**

*Hafnia alvei* is a member of the family *Enterobacteriaceae* and can be isolated from various anatomical sites in humans and from various environmental sources (8). A study in Japan has reported that it could be isolated from approximately 13% of fecal specimens from normal healthy individuals (11), and to our knowledge it has never been implicated as a causative agent of diarrhea.

In this study, we report the isolation of *H. alvei* from a child with watery diarrhea and demonstrate by laboratory investigations a virulence attribute which makes at least some strains of *H. alvei* potential enteropathogens.

## MATERIALS AND METHODS

**Case.** A 9-month-old girl with vomiting and diarrhea was brought to the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) for treatment. The diarrhea, which lasted 3 days, was accompanied by vomiting, mild dehydration, fever, and distention of the abdomen. Stools appeared liquid, yellow, and without mucus or blood. Other signs and symptoms were unremarkable. She was treated with rice-based oral rehydration fluid and discharged 20 h after admission.

Diarrheal stool cultured for enteric bacterial pathogens did not yield any recognized bacterial pathogens, but *H. alvei* was isolated as a pure culture. Parasites and viruses were not sought in the diarrheal stool.

**Identification of *H. alvei*.** The identification of a non-lactose-fermenting colony on MacConkey agar was accomplished by a set of biochemical reactions outlined by Ewing (8) and by reaction in the biochemical strip API-20E for the family *Enterobacteriaceae* (API System, Montalieu, Vercieu, France).

**Enterotoxigenicity assay.** *H. alvei* was grown in Trypticase soy broth supplemented with 0.6% yeast extract (TSBY) (GIBCO) for 20 h with shaking. Cell-free culture filtrates and polymyxin B extracts of cell pellets (9) were tested in mouse adrenal tumor Y-1 cells for heat-labile toxin (17), in suckling mice for heat-stable toxin (6), and in HeLa cells for Shiga-like toxins (3).

**Enteroinvasiveness assay.** The strain was tested for invasiveness in HeLa cells (7) and in a guinea pig's eye by the Sereny test (18).

**Enteroadherence assay.** Adherence to HeLa cells was tested according to the method of Cravioto et al. (5). Briefly, HeLa cell monolayers were inoculated with overnight bacterial culture grown in Luria broth, and they were then incubated for 3 h in the presence of 0.5% D-mannose, after which they were fixed in 70% methanol and stained with Giemsa stain.

**Plasmid analysis.** Plasmid DNA extraction was carried out according to the method of Birnboim and Doly (2). After electrophoresis, the gel was stained with ethidium bromide and examined under UV light.

**RITARD assay.** All animals used in this assay were treated thrice, each time for 5 days, with metronidazole (125 mg per rabbit per day) and sulfaquinoxaline sodium (464 mg per rabbit per day) to clear the gut of giardias and coccidia, respectively; only giardia- and coccidium-free animals were used for experiments. The ability of *H. alvei* to cause diarrhea was tested in 12 rabbits with removable intestinal ties (RITARD model) (19). Abdominal surgery was performed after the rabbits had fasted for 24 h, and a permanent tie was introduced into the cecum. Ten-milliliter aliquots of an overnight culture of *H. alvei* in TSBY, each aliquot containing $10^{10}$ organisms, were injected into the intestine

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TABLE 1. Fecal shedding, diarrhea, and colonization of the gut in rabbits with removable intestinal tie inoculated with *H. alvei*

| No. of rabbit | Diarrhea[a] (day after inoculation) | Postinoculation shedding of organism in stool[b] | No. of bacteria/g of mucosal scraping of: | | Histological evidence of bacterial adherence in: | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mid-jejunum | Mid-ileum | Ileum | Cecum | Colon | Rectum |
| 1 | + (1) | H, E (5) | ND[c] | ND | ND | ND | ND | ND |
| 2 | + (3) | H, E (3) | $1 \times 10^6$ (H) | $3 \times 10^5$ (H) | + | + | + | + |
| 3 | − | H (4) | ND | ND | − | − | + | − |
| 4 | + (2) | H (3) | ND | ND | ND | ND | ND | ND |
| 5 | + (2) | H (10) | ND | ND | ND | ND | ND | ND |
| 6 | + (4) | H (10) | $1 \times 10^5$ (H) | $3 \times 10^4$ (H) | ND | ND | ND | ND |
| 7 | + (3) | H, E (4) | $1 \times 10^8$ (H), $2 \times 10^5$ (E) | $6.5 \times 10^8$ (H), $5 \times 10^7$ (E) | ND | ND | ND | ND |
| 8 | − | H, E (10) | | | ND | ND | ND | ND |
| 9 | + (2) | H, E (5) | $1 \times 10^4$ (H) | $4.3 \times 10^5$ (H) | + | + | − | − |
| 10 | − | H, E (4) | $4 \times 10^3$ (E) | $9 \times 10^3$ (H) | − | − | + | − |
| 11 | + (2) | H (4) | $8 \times 10^4$ (H) | $4 \times 10^6$ (H) | + | + | + | − |
| 12 | − | H, E (5) | $5 \times 10^4$ (E) | $2.9 \times 10^4$ (E), $7 \times 10^4$ (H) | − | − | − | − |

[a] +, Presence of diarrhea; −, absence of diarrhea.
[b] H, *H. alvei*; E, *E. coli*. Number in parentheses indicates day of sacrifice; shedding of indicated organism occurred until that day.
[c] ND, Not done.

above the temporary small intestinal tie, and the tie was removed 2 h later. Control experiments were performed on eight rabbits with *Escherichia coli* K-12. Unless stated otherwise, the animals were observed for 5 days for diarrhea and death. Rectal swabs were taken daily and plated onto MacConkey agar to monitor shedding of the challenge organism. The animals were sacrificed at various periods after challenge when they developed diarrhea. However, all control animals which did not develop diarrhea (see results below) were sacrificed at the end of the fifth day. At autopsy, the intestines were examined for fluid accumulation and other gross pathological changes. Intestinal sections were taken from the upper and lower jejunum, ileum, cecum, proximal and distal colon, appendix, and mesenteric lymph nodes, and they were fixed in buffered formal saline (21). Mucosal scrapings of the mid-jejunum and mid-ileum were cultured quantitatively. Serial 10-fold dilutions of homogenized tissue were prepared in sterile physiological saline, plated on MacConkey agar, and incubated at 37°C for 24 h.

**Oral feeding of rabbits.** Five adult rabbits which had fasted for 24 h were given 15 ml of a 5% sodium bicarbonate solution via a gastric tube to neutralize gastric acidity. This was followed by an oral challenge with 10 ml of a culture of *H. alvei* containing $10^{10}$ bacteria for two rabbits and with *E. coli* K-12 culture for the other three rabbits. The animals were observed daily for development of diarrhea, and shedding of the organisms was monitored as described above.

**Rabbit ileal loop assay.** Either 1 ml of a 20-h TSBY culture containing $10^9$ bacteria or cell-free culture filtrates were inoculated into the approximately 10-cm-long intestinal ileal loops of four adult New Zealand White rabbits which had previously fasted for 48 h. Not more than six loops were made in each rabbit. After 20 h, rabbits were sacrificed and loops were examined for fluid accumulation and other gross pathological changes (12). Portions of loops were fixed in buffered formal saline and processed for histopathology.

## RESULTS

The organism was positive for the following tests: o-nitrophenyl-β-D-galactopyranoside, lysine decarboxylase, ornithine decarboxylase, and the fermentation of glucose, mannitol, and arabinose. It was negative for the following tests: oxidase; arginine dihydrolase; tryptophan deaminase; citrate utilization; $H_2S$, urease, and indole production; methyl red; Voges-Proskauer; gelatin liquefaction; and the fermentation of inositol, sorbitol, rhamnose, saccharose, melibiose, and amylose.

The organism was negative for the following virulence properties: heat-labile and heat-stable enterotoxins, Shiga-like toxins, and enteroinvasiveness in the HeLa cell assay (survival of bacteria, 0%) and by the Sereny test; it also failed to adhere in the HeLa cell assay. In addition, no plasmid could be identified by gel electrophoresis.

**RITARD assay.** All 12 rabbits inoculated with *H. alvei* shed the organism in the feces either alone or mixed with *E. coli*. Eight of them developed diarrhea between 1 and 4 days after inoculation; the diarrhea lasted in all animals until they were sacrificed (Table 1). Feces were soft, mucoid, and without blood, which soiled the perianal region of the affected rabbits. None of the eight rabbits inoculated with *E. coli* K-12 developed diarrhea.

At autopsy, rabbits with diarrhea had moderate amounts of greenish yellow, mucoid fluid in the lower part of the small intestine and substantial amounts in the portion of the cecum that remained patent.

Quantitative bacteriology was performed on the gut scrapings from the jejuna and mid-ilea of five rabbits with diarrhea (rabbit numbers 2, 6, 7, 9, and 11) and two rabbits without diarrhea (numbers 10 and 12). Four rabbits with diarrhea had pure growth of *H. alvei* in both the jejunum and ileum, and the fifth had a mixture of *E. coli* and *H. alvei*. The number of *H. alvei* in the jejunum ranged between $9 \times 10^3$ and $6.5 \times 10^8$ per g of mucosal scraping. In the two rabbits which did not have diarrhea, only *E. coli* was cultured from the jejunum, with only *H. alvei* cultured from the ileum of rabbit 10 and *H. alvei* and *E. coli* cultured from the ileum of rabbit 12. The number of *H. alvei* in the ilea of these rabbits ranged between $9 \times 10^3$ and $7 \times 10^4$ per gm of mucosal scraping.

The histologies of rabbits 2, 9, and 11, which developed diarrhea and contained only *H. alvei* in the mid-jejunum and mid-ileum, showed bacterial attachment in the lower ilea of all three (Fig. 1). All three rabbits had evidence of bacterial attachment in the portions of the cecum that remained patent; in addition, attachment of bacteria was seen in the colon and rectum in rabbit 2 and the colon in rabbit 11. Pathological changes in the small intestine included erosion of surface epithelium; dilatation of lacteals in the villi; and

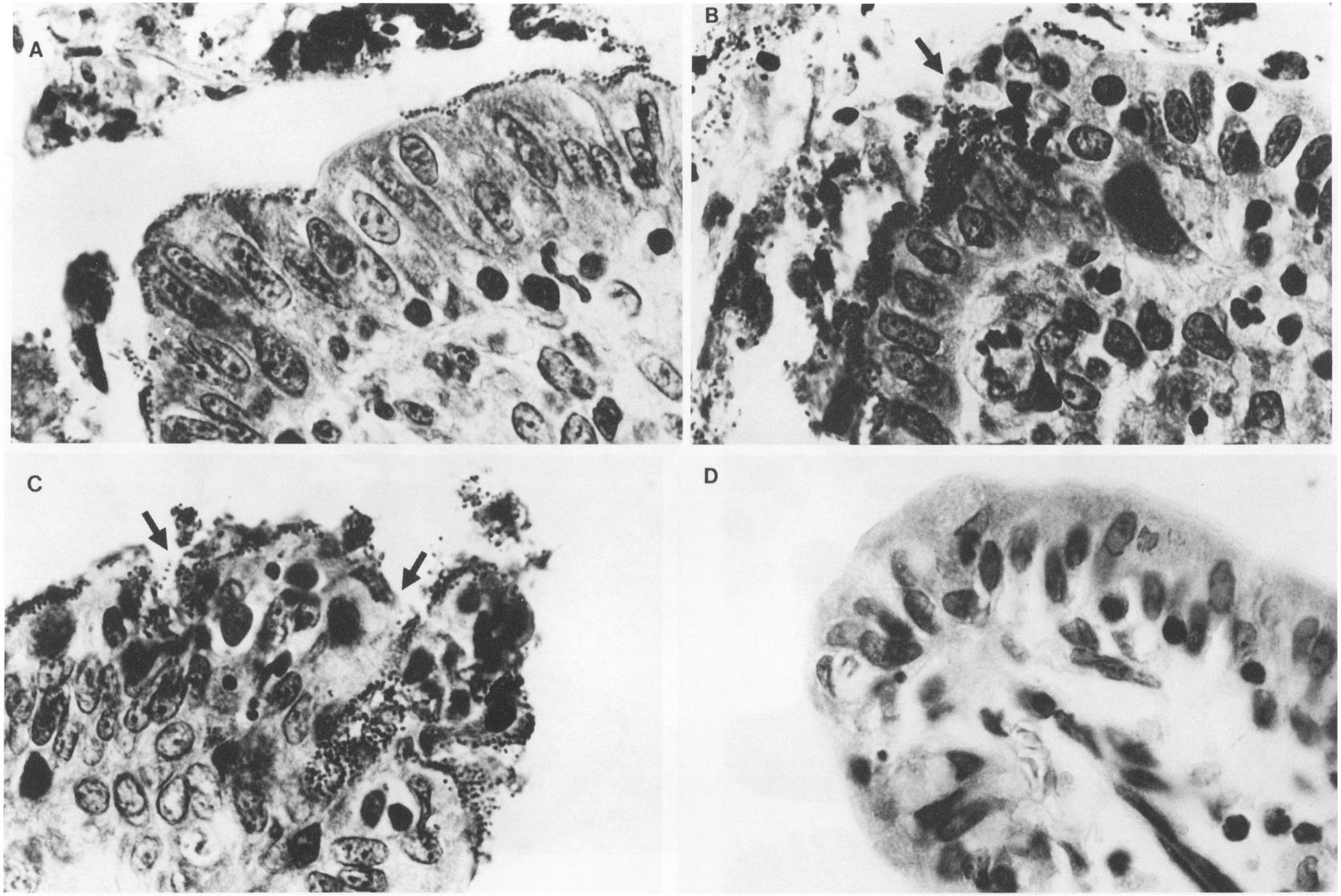
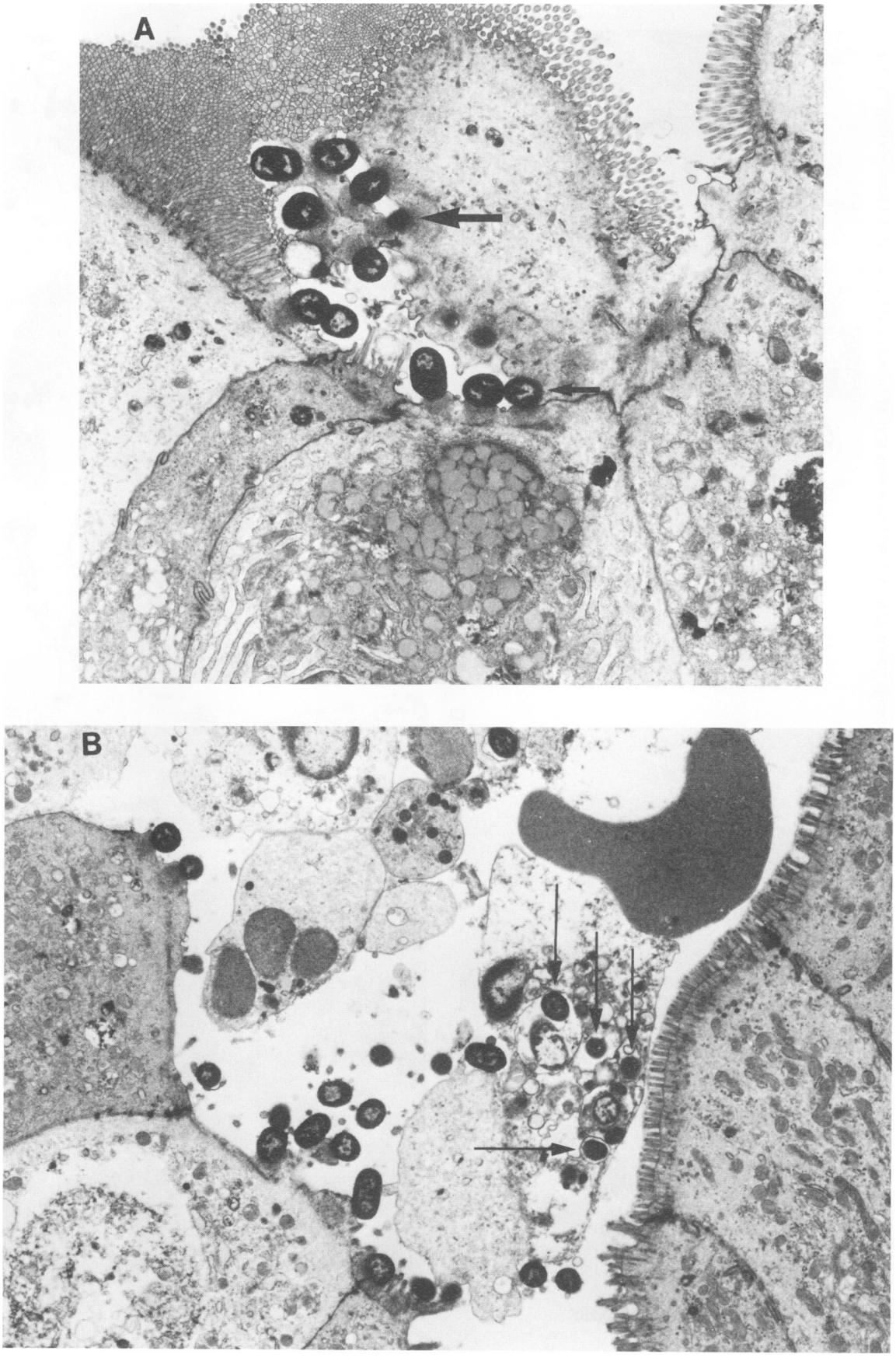



FIG. 1. Ileal sections (A, B, and C) from rabbit 11 48 h after inoculation with *H. alvei*, showing successive stages of epithelial surface injury, compared with a section taken from an animal inoculated with *E. coli* K-12 (D). (A) Attachment of bacteria to as yet intact enterocytes, with several inflammatory cells appearing at the basement membrane; (B) a progressive cell-bacterium interaction, with evidence of a beginning of surface erosion (arrow); (C) surface epithelium is irregular, eroded in several locations (arrows), and with bacteria invading dead or dying cells. Note inflammatory cellular infiltration into injured sites (magnification, ×430; hematoxylin and eosin stain).


A
B

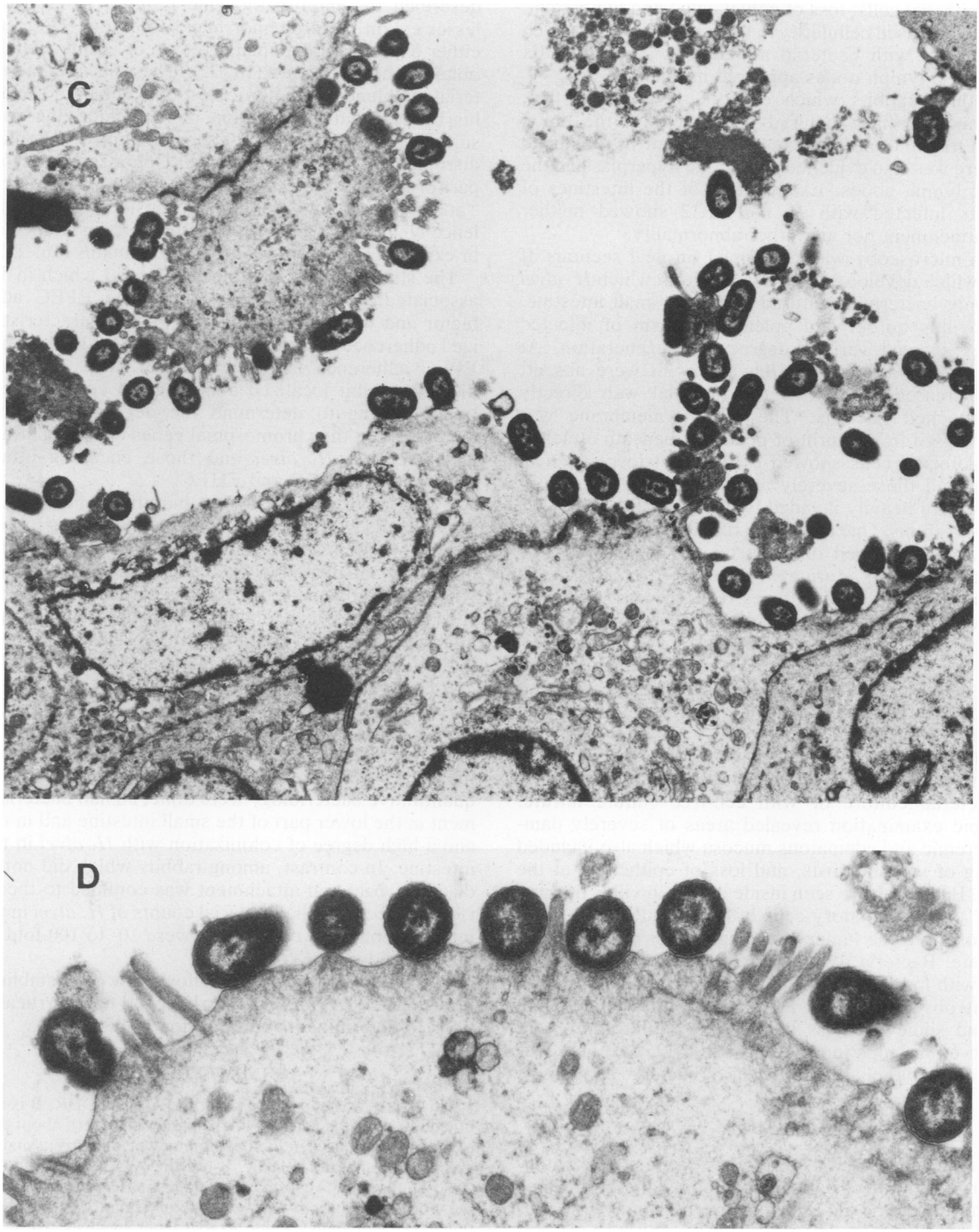



FIG. 2. Ultrathin ileal sections (A, B, C, and D) from rabbit 11 48 h after inoculation with *H. alvei*, showing various stages of cell surface-bacterium interface. (A) Bacteria are seen propagating (by bacterial cell division) (small arrow). Note that cells and microvillus border are still intact (magnification, ×8,000). (B) Affected cells with attached bacteria and disrupted cellular organelles can be seen on the left side of the micrograph, and unaffected cells with intact microvillus border can be seen on the right. Portion of a dead cell heavily invaded by bacteria (arrows) is seen in the lumen (magnification, ×6,470). (C) A villus surface is severely altered; attaching bacteria have effaced the microvillus border and caused the loss of most of the cytoplasm. Bacteria still attached to cellular debris can be seen in the lumen (magnification, ×6,470). (D) High magnification (×15,000) of apical plasma membrane showing cupping or pedestal formation beneath attached bacteria and increased density of the terminal web.

hyperemia, edema, and an increased number of leukocytes in the lamina propria. The cecum and colon showed hyperplasia of epithelial cells; loss of goblet cells; and hyperemia, edema, and increased cellularity in the lamina propria, which were associated with bacterial attachment. The appendix and mesenteric lymph nodes appeared normal.

Of the three rabbits which did not develop diarrhea, rabbits 3 and 10 had bacterial attachment in the colon whereas in rabbit 12 no attachment was seen. In rabbits 10 and 12, there was also evidence of reactive hyperplasia in the mesenteric lymph nodes. Examination of the intestines of two rabbits infected with *E. coli* K-12 showed neither bacterial attachment nor any other abnormality.

Electron microscopy was performed on ileal sections of rabbit 11, which developed diarrhea and from which *H. alvei* was the only bacterium cultured from the small intestine. The microvillus border and apical cytoplasm of infected enterocytes showed various degrees of degeneration. At sites of bacterial attachment, the microvilli were absent, with an increased density of the terminal web directly beneath attached bacteria. The plasma membrane was cupped or raised in the form of pedestals beneath attached bacteria. Affected cells showed evidence of loss of apical cytoplasm, and those severely affected showed extrusion. Dead cells were heavily invaded by bacteria (Fig. 2).

**Orally fed rabbits.** One of the two rabbits orally challenged with *H. alvei* developed diarrhea on the fourth day; the diarrhea lasted 3 days, during which time *H. alvei* was the only organism cultured from feces. Before diarrhea developed, after diarrhea stopped, and until the last day of observation (14th day), it shed a mixture of *E. coli* and *H. alvei*. The other rabbit, which did not develop diarrhea, shed a mixture of *E. coli* and *H. alvei* throughout the 2 weeks of observation. None of three rabbits challenged with *E. coli* K-12 developed diarrhea.

**Rabbit ileal loop assay.** Inoculation of four loops in each of four rabbits did not cause fluid accumulation with either whole bacterial culture or with cell-free culture filtrate. Microscopic examination revealed areas of severely damaged hyperemic and edematous mucosa which also included broadening of villi, necrosis, and loss of epithelium at the villus tips. Bacteria were seen inside damaged villus tips and "streams" of inflammatory cells often extended from damaged mucosa into the lumen, forming a layer of mucopurulent exudate. Bacteria were seen attached to epithelial cell surfaces, with loss of microvillus border in a manner identical to that observed in the RITARD rabbits. Hyperemia, edema, and somewhat less inflammatory infiltration were also observed in loops inoculated with K-12, but there was no evidence of epithelial cell necrosis or bacterial adherence.

## DISCUSSION

*H. alvei* was suspected as a possible cause of the watery diarrhea in this child because it was the only organism which was cultured and was in abundance in the stool. However, *H. alvei* is not known to cause diarrhea, and when it was tested, our isolate did not possess the conventional virulence properties of either toxin production or invasiveness.

The RITARD model has previously been used as a suitable model to investigate the diarrheagenic properties of a variety of organisms (15, 19). *H. alvei* produced diarrhea in 8 of 12 rabbits and in 1 of 2 orally inoculated rabbits. In both models, organisms colonized the gut, resulting in the excretion of bacteria in feces, sometimes with total displacement of normal flora.

Light and electron microscopy showed mucosal lesions which were indistinguishable from those which have been described for enteropathogenic *E. coli* (EPEC) and, to a lesser extent, enterohemorrhagic *E. coli* (EHEC) strains in either experimental animals (12, 20) or in intestinal biopsies obtained from patients (16, 22). These lesions include bacterial attachment to enterocytes, effacement of the microvillus border, and modification of the underlying apical cell surface, which often lead to loss of apical cytoplasm and disruption of cellular organelles. Colonization of the major part of the small intestine and often the large intestine with "attaching-effacing" bacteria is considered the major virulence characteristic by which EPEC strains induce diarrhea in experimental animals (20) and in humans (16, 22).

The strain appeared to lack a plasmid which in EPEC is associated with the expression of the EPEC adherence factor and which is identifiable by the characteristic localized adherence in HeLa or HEp-2 cells (1, 10, 14) and by the EPEC adherence factor probe (13). The *H. alvei* strain did not exhibit localized adherence in cell culture. It will be interesting to determine the degree of homology, if any, between the chromosomal genes encoding attachment-effacement of *H. alvei* and those encoding attachment-effacement of EPEC and EHEC.

It was important to establish that the rabbits used in these experiments were free from naturally occurring rabbit EPEC strains (4). This was clear, as diarrhea occurred in rabbits only when inoculated with *H. alvei* and not when inoculated with nonpathogenic *E. coli* K-12; characteristic lesions were seen in rabbits which had only *H. alvei* isolated from the gut and were also seen in the ileal loops of rabbits inoculated with *H. alvei* and not in those of rabbits inoculated with *E. coli* K-12.

As was demonstrated in the piglet model for EPEC and EHEC (20), the occurrence of diarrhea in rabbits was influenced by the degree and site of bacterial attachment in the gut. Of the three diarrheal rabbits in which histology and quantitative bacteriology were done, all had bacterial attachment in the lower part of the small intestine and in the colon and a high degree of colonization with *H. alvei* in the small intestine. In contrast, among rabbits which did not develop diarrhea, bacterial attachment was confined to the colon in two of three animals. Bacterial counts of *H. alvei* in the small intestines of these two rabbits were 10- to 100-fold less and were mixed with *E. coli*.

This study should now be followed by one establishing the prevalence of *H. alvei* in diarrheal and nondiarrheal populations to determine its contribution to disease.

## ACKNOWLEDGMENTS

This study was supported by ICDDR, B. ICDDR, B is supported by countries and agencies which share its concern about the impact of diarrheal diseases in the developing world. Current major donors giving assistance to ICDDR, B are the Aga Khan Foundation, the Arab Gulf Fund, Australia, Bangladesh, Belgium, Canada, IDRC, the Ford Foundation, Japan, the Norwegian Agency for International Development, Saudi Arabia, the Swedish Agency for Research Co-operation with Developing Countries, Switzerland, the United Kingdom, UNICEF, UNDP, USAID, and WHO.

We thank Priyatosh Sukul for typing the manuscript.

## REFERENCES

1. **Baldini, M. M., J. B. Kaper, M. M. Levine, D. C. A. Candy, and H. W. Moon.** 1983. Plasmid-mediated adhesion in enteropathogenic *Escherichia coli*. J. Pediatr. Gastroenterol. Nutr. **2**:534–538.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction

procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 1:1513–1523.
3. **Bitzan, M., H. Karch, H. Altrogge, J. Strehlau, and F. Blaker.** 1988. Hemolytic-uremic syndrome associated with a variant Shiga-like cytotoxin of *Escherichia coli* O111. Pediatr. Infect. Dis. J. 7:128–132.
4. **Cantey, J. R., and R. K. Blake.** 1977. Diarrhea due to *Escherichia coli* in the rabbit: a novel mechanism. J. Infect. Dis. 135:454–462.
5. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. 3:95–99.
6. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. 125:407–411.
7. **Donnenberg, M. S., A. Donohue-Rolfe, and G. T. Keusch.** 1989. Epithelial cell invasion: an overlooked property of enteropathogenic *Escherichia coli* (EPEC) associated with the EPEC adherence factor. J. Infect. Dis. 160:452–459.
8. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed., p. 417–421. Elsevier Science Publishing Co., Inc., New York.
9. **Griffin, D. F., and P. Gemski, Jr.** 1983. Release of Shiga toxin from *Shigella dysenteriae* 1 by polymyxin B. Infect. Immun. 40:425–428.
10. **Knutton, S., M. M. Baldini, J. B. Kaper, and A. S. McNeish.** 1987. Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells. Infect. Immun. 55:78–85.
11. **Matsumoto, H.** 1963. Studies on the *Hafnia* isolated from normal human. Jpn. J. Microbiol. 7:105–114.
12. **Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Gianella.** 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infect. Immun. 41:1340–1351.
13. **Nataro, J. P., M. M. Baldini, J. B. Kaper, R. E. Black, N. Bravo, and M. M. Levine.** 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J. Infect. Dis. 152:560–565.
14. **Nataro, J. P., I. C. A. Scaletsky, J. B. Kaper, M. M. Levine, and L. Trabulsi.** 1985. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect. Immun. 48:378–383.
15. **Pazzaglia, G., R. B. Sack, A. L. Bourgeois, J. Froehlich, and J. Eckstein.** 1990. Diarrhea and intestinal invasiveness of *Aeromonas* strains in the removable intestinal tie rabbit model. Infect. Immun. 58:1924–1931.
16. **Rothbaum, R. J., J. C. Partin, K. Saalfield, and A. J. McAdams.** 1983. An ultrastructural study of enteropathogenic *Escherichia coli* infection in human infants. Ultrastruct. Pathol. 4:291–304.
17. **Sack, D. A., and R. B. Sack.** 1975. Test for enterotoxigenic *Escherichia coli* using Y1 adrenal cells in miniculture. Infect. Immun. 11:334–336.
18. **Sereny, B.** 1957. Experimental keratoconjunctivitis shigellosa. Acta Microbiol. Acad. Sci. Hung. 4:367–376.
19. **Spira, W. M., R. B. Sack, and J. L. Froehlich.** 1981. Simple adult rabbit model for *Vibrio cholerae* and enterotoxigenic *Escherichia coli* diarrhea. Infect. Immun. 32:739–749.
20. **Tzipori, S., R. Gibson, and J. Montanaro.** 1989. Nature and distribution of mucosal lesions associated with enteropathogenic and enterohemorrhagic *Escherichia coli* in piglets and the role of plasmid-mediated factors. Infect. Immun. 57:1142–1150.
21. **Tzipori, S., R. M. Robins-Browne, G. Gonis, J. Hayes, M. Withers, and E. McCartney.** 1985. Enteropathogenic *Escherichia coli* enteritis: evaluation of the gnotobiotic piglet as a model of human infection. Gut 26:570–578.
22. **Ulshen, M. H., and J. L. Rollo.** 1980. Pathogenesis of *Escherichia coli* gastroenteritis in man—another mechanism. N. Engl. J. Med. 302:441–454.