INFECTION AND IMMUNITY, Jan. 1982, p. 305-313
0019-9567/82/010305-09$02.00/0

Vol. 35, No. 1

Pilus Production, Hemagglutination, and Adhesion by Porcine Strains of Enterotoxigenic *Escherichia coli* Lacking K88, K99, and 987P Antigens

M. AWAD-MASALMEH,† H. W. MOON,* P. L. RUNNELS, AND R. A. SCHNEIDER

National Animal Disease Center, U.S. Department of Agriculture, Science and Education Administration, Ames, Iowa 50010

Received 20 July 1981/Accepted 21 August 1981

Three strains of enterotoxigenic *Escherichia coli* which adhered, colonized intensively, and caused disease in pig intestine, but which did not produce pili of the K88, K99, or 987P antigen types were designated 3P⁻ ETEC. The 3P⁻ ETEC caused mannose-resistant hemagglutination, adhered to porcine intestinal epithelial cells in vitro, and produced pili. However, most bacteria taken directly from the intestine of pigs infected with 3P⁻ ETEC appeared to be nonpiliated. Two preparations were isolated from the 3P⁻ ETEC. One (material A) contained pili, caused mannose-sensitive hemagglutination, and did not inhibit adhesion of whole bacteria to epithelial cells in vitro. The other (material B) had no demonstrable pili, caused mannose-resistant hemagglutination, and blocked ahesion of bacteria to epithelial cells in vitro. Antiserum against an acapsular mutant (K⁻) of one 3P⁻ ETEC strain was absorbed to remove antibodies directed against somatic (O) antigen. The absorbed antiserum agglutinated all three 3P⁻ ETEC strains grown in the K⁻ form at 37°C, but not when they were grown at 18°C. The absorbed antiserum blocked the hemagglutinating activity of material B, but not of material A. It also reacted (via indirect immunofluorescence) with all of the 3P⁻ ETEC when they were grown in pig intestine. The results were interpreted to indicate that: (i) the epithelial adhesive and mannose-resistant hemagglutinating activities of the 3P⁻ ETEC strains may be mediated by an antigen contained in material B; (ii) this antigen either is not pilus associated or is associated with pili that are not demonstrable by the methods used here; (iii) the 3P⁻ ETEC strains produce type 1 pili which do not mediate their adhesion to intestinal epithelium of pigs.

Pilus-mediated adhesion to epithelium appears to be essential for colonization of the small intestine by enterotoxigenic *Escherichia coli* (ETEC). However, pili apparently are only one of a constellation of attributes which facilitate colonization by ETEC. For example, both capsule and pili are required for colonization by some strains of ETEC (8, 13, 15, 16). Furthermore, some ETEC that do not produce any of the pili that have been implicated in intestinal colonization are enteropathogenic, i.e., they colonize intestine and cause diarrhea in pigs (11) or people (9). Three antigenic types of pili (K88, K99, and 987P) have been implicated in colonization of pig intestine by ETEC. ETEC that adhered, colonized, and caused disease in pig small intestine, without producing any of these three types of pili, were designated as 3P⁻ ETEC (11).

The hypothesis of this study was that 3P⁻ ETEC produce previously unrecognized antigenic types of pili which mediate their intestinal adhesion and colonization. We found that the 3P⁻ ETEC do produce pili, but the pili demonstrated apparently do not mediate intestinal colonization and adhesion. We present evidence that adhesion of 3P⁻ ETEC to intestinal epithelium is mediated by material which does not contain pili demonstrable by electron microscopic examination of negatively stained preparations.

MATERIALS AND METHODS

E. coli strains and growth conditions. The 10 *E. coli* strains used and their characteristics are listed in Table 1. Previous tests demonstrated that the 3P⁻ strains did not produce K88, K99, or 987P antigens (11). Doyle Evans (personal communication) determined that they did not produce pilus antigens CFA/I or CFA/II (4). Acapsular (K⁻) mutants were selected by methods reported previously (13). Unless stated otherwise, all results were from bacteria grown on Minca agar plus IsoVitaleX (BBL Microbiology Systems; Minca Is) (5) at 37°C for 24 h. For some studies, bacteria were

† Visiting scientist from the Institute of Bacteriology and Veterinary Hygiene, University of Veterinary Medicine, Vienna, Austria.

TABLE 1. Characteristics of the *E. coli* strains used

Strain no.	Source	Serotype	Enterotoxins[a]	Pilus antigen
VAC-1676	Swine	O101:K30:H$^-$ [b]	STa	3P$^-$ [c]
KATI-1706	Swine	O101:K30:H$^-$ [b]	STa	3P$^-$
VC-1751	Swine	O101:K27:H$^-$ [b]	STa	3P$^-$
431	Swine	O101:K30:H$^-$	STa	K99
637	Swine	O64:K+:H$^-$	STa	K99
987	Swine	O9:K103:H$^-$	STa	987P
263	Swine	O8:K87:H19	LT	K88
123	Swine	O43:K$^-$:H28	None	?
1793	Laboratory[d]	O9:K30:H1	None	?
1788	Laboratory	O101:K$^-$:H$^-$	None	?

[a] STa is enterotoxin demonstrable in infant mice, and LT is enterotoxin demonstrable in adrenal cells in culture.
[b] Determined by Fritz and Ida Ørskov.
[c] Does not produce K88, K99, or 987P, but does colonize pig intestine.
[d] Standard laboratory strain, original source not known.

grown on blood agar base (Difco) plus 5% sheep blood or on Trypticase soy agar (BBL) at 37°C. When desired, pellicle formation was encouraged by growth at 37°C for up to a week in Trypticase soy broth (BBL).

Electron microscopy. Cultures and fluids examined for pili by electron microscopy were prepared, using the potassium phosphotungstate method for negative staining, and examined as reported previously (8). Sections of ileum from neonatal colostrum-deprived pigs were obtained 16 to 18 h after the pigs were infected with ETEC. The sections were prepared and examined by electron microscopy (12).

Hemagglutination. Hemagglutination tests were conducted as described by Evans et al. (4).

Adhesion to intestinal epithelial cells in vitro. Adhesion determinations were conducted by a microscopic method (14). The epithelial cells used were isolated from the small intestine of hysterectomy-derived, colostrum-deprived pigs that were less than 12 h old.

Antiserum production. A K$^-$ mutant of strain KATI-1706 was grown on Minca Is and then inoculated into broth for antigen production. The antigen produced was used to raise antiserum in rabbits. Antigen production and immunization were done according to the procedure cited as method 1 for the production of *E. coli* O:K antiserum (3). The antiserum produced was absorbed with the K$^-$ mutant of strain KATI-1706, grown at 18°C. The serum was first absorbed with this antigen after the antigen had been heated (100°C, 1 h), and the serum was then absorbed with the living bacteria. These absorptions were to remove O antibodies and antibodies against surface antigens produced when the strain was grown at 18°C. Serum absorption procedures and agglutination tests (slide method) were done by standard techniques (3). The absorbed serum did not agglutinate appropriately prepared O101 or K30 antigens but did agglutinate (titer of 1:200, slide agglutination) the autologous living acapsular mutant grown on Minca Is agar at 37°C. The agglutinins that remained in the absorbed antiserum were assumed to be directed against surface antigens, and the absorbed antiserum will be referred to as 1706-SA (surface antigens).

Immunofluorescence. Sections of ileum from newborn colostrum-deprived pigs were obtained 16 to 18 h after the pigs were infected with ETEC (13). The sections were prepared and stained by using the 1706-SA antiserum and an indirect immunofluorescent technique (11).

Isolation of materials A and B. Acapsular mutants of strains VAC-1676, KATI-1706, and VC-1751 grown on Minca Is agar for 24 h at 37°C were suspended in phosphate-buffered saline at pH 8.5 (PBS). The suspensions were frozen and thawed twice, agitated vigorously in a Vortex mixer for 15 min, and centrifuged (12,000 × *g* and 4°C for 15 min). The supernatants were harvested, and the centrifugations were repeated to remove debris. Acetic acid was added to the clear supernatants to pH 5.5, and the supernatants were centrifuged (27,000 × *g*, 4°C, 10 min). The resulting pellets were separated from the supernatants and were redissolved in PBS, labeled as material A, and frozen at −70°C. The supernatants were mixed with ammonium sulfate (70% saturation), held for 2 h at 4°C, and then centrifuged (27,000 × *g*, 4°C, 15 min). The resulting pellets were dissolved in PBS, labeled as material B, frozen, and stored at −70°C.

Purification of material A. Material A was thawed, dialyzed in 0.05 M sodium phosphate at pH 7.0 (PO_4 buffer), and centrifuged (48,000 × *g*, 4°C, 15 min). The pellet was discarded, and the supernatant was harvested, brought to 10% saturation with ammonium sulfate, and recentrifuged. The resulting pellet was suspended in PO_4 buffer and applied to a Sephadex G25 column equilibrated with PO_4 buffer. Fractions were collected and assayed for absorbance at 280 nm. The fractions coinciding with the first absorbance peak eluted were pooled and brought to 10% ammonium sulfate saturation. The resulting precipitate was separated by centrifugation, dissolved in PO_4 buffer, dialyzed against PO_4 buffer, and labeled as purified material A.

Purification of material B. Material B was thawed, dialyzed, and centrifuged by the same methods as material A. The resulting supernatant was brought to 60% saturation with ammonium sulfate and centrifuged (48,000 × *g*, 4°C, 15 min). The pellet was harvested, suspended in PO_4 buffer, and applied to a Sephadex G50 column equilibrated with PO_4. Fractions were collected, and those coinciding with the first peak of absorbance at 280 nm eluted were pooled, brought to 20% ammonium sulfate saturation, and

TABLE 2. MRHA[a] caused by enterotoxigenic *E. coli* of different pilus antigen types

E. coli			Hemagglutination of erythrocytes from[b]:						
Strain	Growth medium	Pilus type	Pigs	Sheep	Rabbits	Chickens	Guinea-pigs	Cattle	Horses
VAC-1676	Minca Is agar	3P$^-$ [c]	4+	4+	4+	4+	4+	0	1+
KATI-1706	Minca Is agar	3P$^-$	4+	4+	4+	4+	4+	0	1+
VC-1751	Minca Is agar	3P$^-$	4+	4+	4+	4+	4+	0	1+
431	Minca Is agar	K99	4+	4+	4+	4+	4+	2+	4+
263	Trypticase soy agar	K88	0	1+	2+	2+	2+	0	0
987	Blood agar	987P	0	0	0	1+	0	0	0

[a] Test solutions contained 0.5% mannose.
[b] 4+, Instantaneous and complete hemagglutination; 2+, delayed and incomplete hemagglutination; 1+, weak or questionable hemagglutination.
[c] Does not produce K88, K99, or 987P.

centrifuged (48,000 × *g*, 4°C, 15 min). The supernatant was harvested, brought to 30% saturation with ammonium sulfate, and recentrifuged. The pellet was harvested, suspended in PO_4 buffer, dialyzed against PO_4 buffer, and labeled as purified material B.

Immunodiffusion and electrophoresis. Agar gel double-diffusion (Ouchterlony) and polyacrylamide gel electrophoresis tests were conducted as reported previously (6).

RESULTS

Bacterial hemagglutination. Cultures of the 3P$^-$ strains (VAC-1676, KATI-1706, and VC-1751) grown on Minca Is agar in the K$^+$ parental forms and as K$^-$ mutants all strongly hemagglutinated porcine erythrocytes in a D-mannose-resistant fashion (mannose-resistant hemagglutination; MRHA). This activity was not present when the cultures were grown on Trypticase soy agar or blood agar. Therefore, subsequent hemagglutination tests with the 3P$^-$ strains were done using K$^+$ bacteria grown on Minca Is agar. The 3P$^-$ strains also caused instantaneous and complete MRHA of erythrocytes from sheep, rabbits, chickens, and guinea pigs, but not of erythrocytes from cattle and horses (Table 2). This MRHA pattern of the 3P$^-$ strains was different from the MRHA patterns of K99$^+$, K88$^+$, and 987P$^+$ strains of *E. coli* that were grown to facilitate production of K99, K88, or 987P pili (Table 2).

The MRHA activity of the 3P$^-$ strains for pig erythrocytes was not enhanced by cooling (4°C) and was not reversed when the agglutinated mixtures were warmed to 40, 50, or 60°C. When the 3P$^-$ strains were grown at 18°C instead of 37°C they did not cause MRHA of erythrocytes from pigs.

Pellicle and pilus formation. The 3P$^-$ strains developed pellicles on the surfaces of broth cultures after several days of incubation at 37°C. About 10% of the bacteria from the pellicles and from Minca Is agar were piliated when examined by negative staining and electron microscopy. The pili were straight or gently curved and regular and had a mean diameter of 5.1 nm (Fig. 1).

Serology. The 1706-SA antiserum did not agglutinate appropriately prepared O101, K30, K88, K99, or 987P antigens (prepared from strains 1788, 1793, 263, 637, and 987, respectively). It did agglutinate strains VAC-1676, KATI-1706, and VC-1751 (titer, 1:200 by slide agglutination) when they were grown in the K$^-$ form on Minca Is agar at 37°C. This agglutination did not occur when the bacteria were grown at 18°C on Minca Is agar, or at 37°C on blood or Trypticase soy agar. The K$^+$ forms of VAC-1676, KATI-1706, and VC-1751 were not usually agglutinated by the serum; however, undiluted serum did

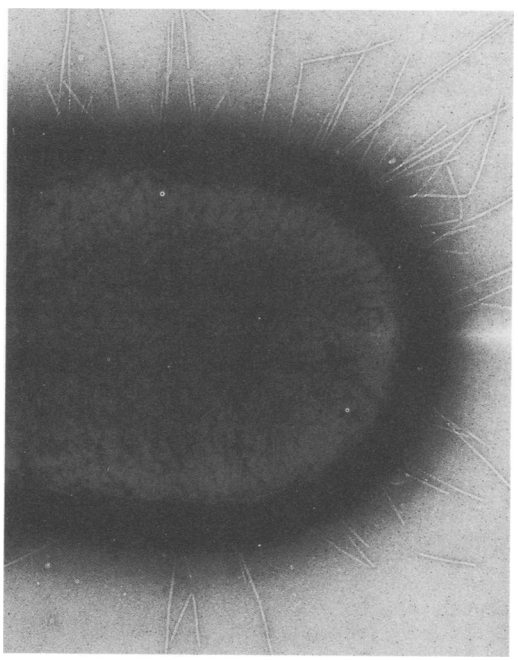

FIG. 1. Electron micrograph of negatively stained *E. coli* VAC-1676. The pili have a mean diameter of 5.1 nm.

sometimes agglutinate them weakly. The agglutinins for KATI-1706 (grown in the K^- form on Minca Is agar at 37°C) were removed by absorbing the serum with either strain VAC-1676 or strain VC-1751 (grown in the K^- form on Minca Is agar at 37°C).

Adhesion to intestinal epithelial cells in vitro. Acapsular mutants of the $3P^-$ strains (Minca Is grown) all adhered to isolated epithelial cells. The number of adhering bacteria per epithelial cell with $3P^-$ strains was somewhat less than that with the $K88^+$ positive control strain 263 but more than that with the negative control strain 123 (Table 3). The adhesion of the $3P^-$ strains was markedly reduced ($\bar{x}$, 2.3 ± 2.8 bacteria per epithelial cell) when the bacteria were grown at 18°C.

Immunofluorescent staining and electron microscopy of bacteria grown in pig ileum. The bacterial layers adherent to ileal villi of pigs infected with each of the $3P^-$ strains (four pigs per strain for each of the three strains) reacted with the 1706-SA antiserum in indirect immunofluorescence (Fig. 2). The serum did not react with the adherent bacterial layer in the ileum of a pig infected with the $987P^+$ strain 987 nor the ilea from any of eight pigs exposed to the nonenterotoxigenic strain 123.

Ileal fluids from the pigs infected with the $3P^-$ strains were prepared (by negative staining) for direct electron microscopic examination. The proportion of the bacteria in these samples that carried demonstrable pili varied from 0 to 2%. The pili observed resembled those produced in vitro (Fig. 1).

Bacterial layers and associated ileal villous epithelium from pigs infected with each of the $3P^-$ strains were examined by electron microscopy (two pigs per strain). The ultrastructural appearance of the layers and epithelium was the same in all six of the pigs. Some, but not all, of the bacteria were surrounded by electron-lucent regions separating the bacteria from each other, as well as from epithelial cell microvilli and luminal debris (Fig. 3 and 4). Much of the vesicular and membranous luminal debris appeared to be from degenerate epithelial cells. The cytoplasm of some epithelial cells was electron lucent, and these cells had patches of distorted or effaced microvilli (Fig. 3). Some epithelial cells had blebs of cytoplasm which appeared to have been extruded through the microvillus border into the intestinal lumen (Fig. 4). The bacterial surfaces had a poorly defined, irregular coat of fuzzy material (Fig. 3), but no discrete attached filaments or pili were demonstrable. There were clumps of unidentified cell-free filaments scattered throughout the bacterial layers (Fig. 3).

Bacterial layers associated with villi from a pig infected with the $K99^+$ strain 431 were also examined by electron microscopy. This strain was chosen for comparison because it has the same O antigen as the $3P^-$ strains and it also has the same capsular (K30) antigen as two of the $3P^-$ strains (Table 1). The appearance of the

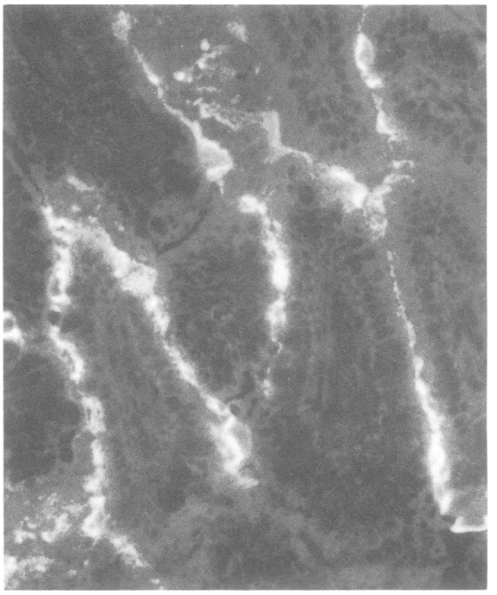

FIG. 2. Immunofluorescent photomicrograph of villi with fluorescent layers of adherent *E. coli* strain KATI-1706. Section from pig ileum stained (indirect method) with antiserum containing antibodies against surface antigens of strain KATI-1706.

TABLE 3. Effects of material A and material B[a] on adhesion of autogenous bacteria to isolated intestinal epithelial cells from pigs

E. coli strain	No. of adherent bacteria per epithelial cell[b] after treatment[c]:		
	None	Material A[d]	Material B
VAC-1676	17.9 ± 10.1	16.8 ± 8.5	5.4 ± 4.2
KATI-1706	14.9 ± 10.2	15.2 ± 11.2	3.4 ± 4.2
VC-1751	20.2 ± 9.7	19.3 ± 10.2	5.2 ± 4.3
263	27.2 ± 6.5	NT	NT
123	7.9 ± 3.2	NT	NT

[a] Materials A and B were extracted from acapsular mutants of *E. coli* strains VAC-1676, KATI-1706, and VC-1751.

[b] Mean ± standard error, microscopic count, 20 epithelial cells evaluated per test and each test replicated five times. NT, Not tested.

[c] Epithelial cells were pretreated with material A or B and then exposed to bacteria of the strain used to prepare the material.

[d] Contains pili demonstrable by negative stain and electron microscopy.

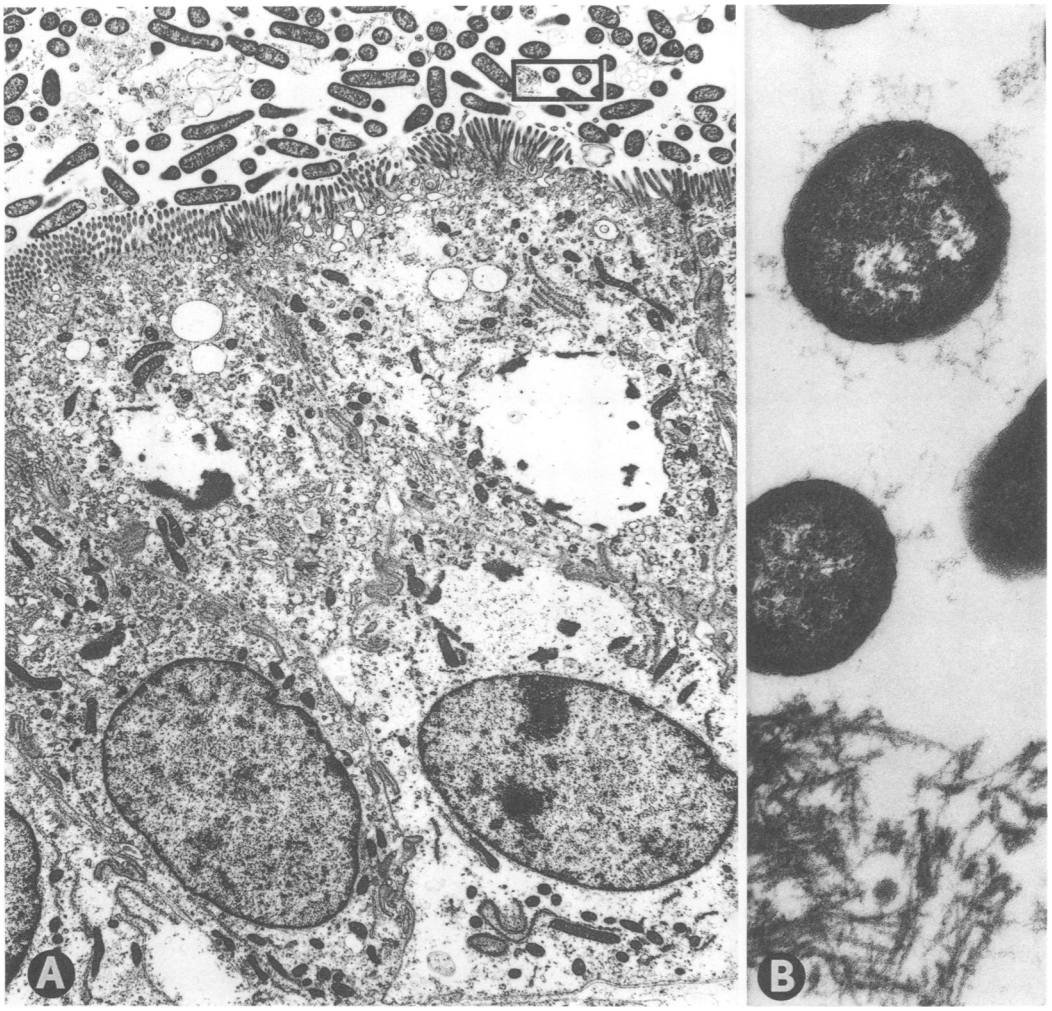

FIG. 3. Electron micrograph of the 3P$^-$ *E. coli* strain KATI-1706 adherent to epithelium on a villus in the ileum of a pig. (A) The bacterial layer contains cell-free filamentous material as well as membranous and vesicular debris. Some of the epithelial cells have patches of irregular or effaced microvilli. Epithelial cells have pale cytoplasm, and the limiting membranes of their large cytoplasmic vacuoles are disrupted. (B) Higher magnification from the area outlined in (A). Cell-free filamentous material at the bottom; bacteria with a poorly defined, irregular coat of fuzzy material above.

layers formed by this strain was as reported previously for this and other K99$^+$ ETEC (1, 10, 12). The layers formed by strain 431 differed from those formed by the 3P$^-$ strains in that electron-lucent regions surrounding bacteria were broader and were associated with almost every bacterium (Fig. 5). Furthermore, nearly all bacteria of strain 431 had a dense fuzzy coat of irregular appendages which at high magnification could be shown to extend across the electron-lucent regions to adjacent bacteria and microvilli (Fig. 5).

Characteristics of material A. Material A from each of the 3P$^-$ strains contained numerous pili (Fig. 6) resembling those demonstrated on the bacterial cells. Material A from each of the three strains hemagglutinated guinea pig erythrocytes. However, in contrast to the MRHA activities of the bacteria (Table 2), the hemagglutinating activities of all three material A preparations were mannose sensitive. The hemagglutinating activity of the material A preparations was not inhibited when the preparations were mixed with 1706-SA antiserum before the tests were done. None of the material A preparations formed precipitin lines when immunodiffusion tests were done using 1706-SA antiserum.

Characteristics of material B. In contrast to material A, we were unable to demonstrate pili by electron microscopic examination of nega-

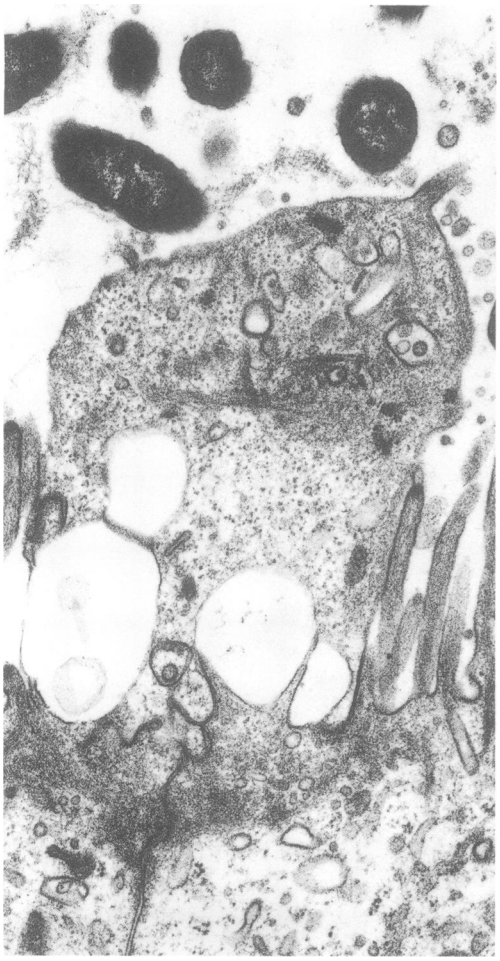

FIG. 4. Electron micrograph of the 3P⁻ *E. coli* strain VC-1751 adherent to epithelium on a villus in the ileum of a pig. A bleb of epithelial cell cytoplasm extrudes through the microvillus border into the lumen. Bacteria with electron-lucent regions between them at the top; intact microvilli towards lower right; epithelial cell junctional complex at the lower left.

tively stained material B from any of the 3P⁻ strains. Furthermore, material B caused MRHA of guinea pig erythrocytes. The MRHA activity of material B was destroyed by heating to 85°C for 1 min or by treatment with 1706-SA antiserum. The activity was not destroyed by antiserum directed against K88, K99, or 987P antigens.

The hemagglutination patterns of purified materials A and B from strain VAC-1676 were compared (Table 4). The pattern for purified material B was the same as that for intact bacterial cells (Table 2).

Material B from each of the three strains and 1706-SA antiserum formed single precipitin lines and lines of identity in immunodiffusion tests. However, precipitin lines did not form in this system when material B preparations and antisera directed against K99, K88, or 987P antigens were used. The mobilities of purified material B and purified K99 were compared in sodium dodecyl sulfate-polyacrylamide gels. Purified material B and K99 both produced single bands. The band produced by purified material B was slightly closer to the origin than that produced by K99, indicating an apparent molecular weight for purified material B slightly greater than that for K99.

Effects of materials A and B on bacterial adhesion to epithelium. Isolated intestinal epithelial cells that were pretreated with material A (0.5 mg of protein per ml at 37°C for 1 h), centrifuged, and washed with PBS remained susceptible to adhesion by bacteria of the 3P⁻ strain used to prepare the material (Table 3). In contrast, such pretreatment with material B markedly inhibited adhesion of bacteria of the autologous strain (Table 3).

DISCUSSION

All of the 3P⁻ ETEC strains produced a cross-reacting surface antigen(s) which was distinct from their somatic O and capsular K antigens and from any of the ETEC pili previously implicated in colonization of pig intestine. This relationship was demonstrated by agglutination of 37°C Minca Is-grown bacteria in 1706-SA antiserum. The relationship was also demonstrated (via indirect immunofluorescence with 1706-SA antiserum) with bacteria grown in pig intestine in vivo. All of these strains also produced large, straight, regular pili which (as material A) caused mannose-sensitive hemagglutination and thus were type 1 pili (common pili or common fimbriae; 4, 18). The 1706-SA antiserum may have contained antibody against these pili. The antigen used to prepare the serum probably contained piliated cells. We don't know whether the 18°C antigen used to absorb the serum contained piliated cells. If 1706-SA antiserum contained antibodies against the type 1 pili they were not in high enough titer or appropriate form to block hemagglutination by material A. The type 1 pili demonstrated were morphologically similar to 987P pili, which have been implicated in intestinal colonization by porcine ETEC (8). However, in contrast to the pili reported here, 987P does not hemagglutinate (8), shifts dramatically to the piliated phase in pig intestine in vivo (11), and blocks adhesion of homologous bacteria to porcine intestinal epithelial cells in vitro (7). The other pili implicated in colonization of pig intestine (K88 and K99) are more irregular (2, 17) than the type 1 pili reported here and they cause MRHA rather than mannose-sensitive

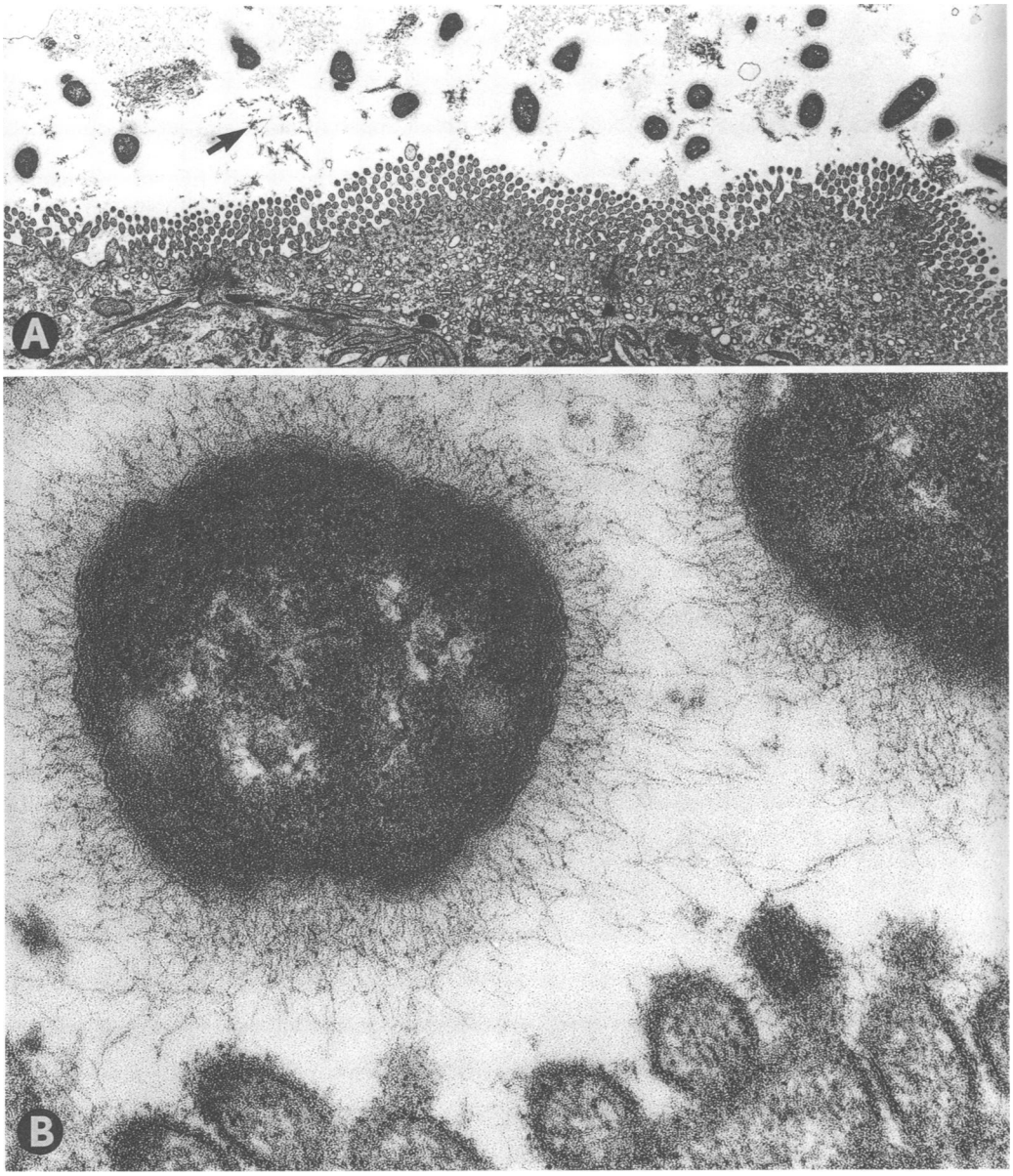

FIG. 5. Electron micrograph of the K99$^+$ *E. coli* strain 431 adherent to epithelium on a villus in the ileum of a pig. (A) Prominent electron-lucent regions separate bacteria from each other and from epithelial cell microvilli. There is cell-free filamentous material (arrow) similar to that shown in Fig. 3. (B) At high magnification, a dense fuzzy coat of irregular appendages extends across the electron-lucent region to adjacent bacteria and to microvilli (at the bottom).

hemagglutination. In contrast to their type 1 pili, bacterial cells of the 3P$^-$ ETEC did cause MRHA. This type of hemagglutination is also characteristic of the pili implicated in colonization of human small intestine by ETEC (4). To date, mannose-sensitive hemagglutinins have not been shown to facilitate colonization of the small intestine by ETEC.

The paucity of type 1 pili on 3P$^-$ ETEC grown in vivo, coupled with the failure of type 1 pili to block adhesion of autologous bacteria to epithelial cells in vitro and their mannose-sensitive hemagglutination, provided evidence that the type 1 pili did not mediate the adhesion of the 3P$^-$ ETEC to pig intestine.

In contrast to material A, material B prepara-

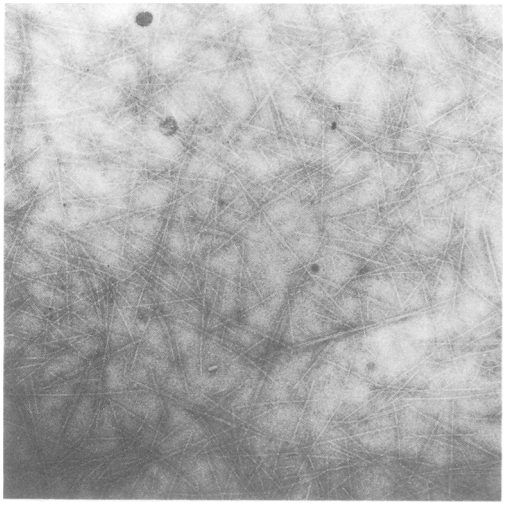

FIG. 6. Electron micrograph of negatively stained pili isolated (material A) from *E. coli* strain VC-1751 (1.5 mg of protein per ml).

tions: (i) did not contain pili, (ii) caused the same MRHA pattern as the autologous bacteria, (iii) did not cause hemagglutination in the presence of 1706-SA antiserum, (iv) formed precipitin lines in immunodiffusion with 1706-SA antiserum, and (v) blocked adhesion of autologous bacteria to intestinal epithelial cells in vitro. Material B preparations contained a substance with an apparent molecular weight comparable to that of K99 in polyacrylamide gel electrophoresis. Two of the $3P^-$ ETEC strains belong to serogroup O101:K30, which frequently contains $K99^+$ ETEC. $K99^+$ ETEC consistently produce a dense coat of irregular filaments (Fig. 5) which presumably contain K99 and which are demonstrable on positively stained bacteria in ultrathin sections, through layers of bacteria adherent to intestinal villi (1, 10, 12). However, the $3P^-$ ETEC did not produce such filaments when examined by this technique. Piliated bacteria from some $K88^+$ and $987P^+$ strains are not demonstrated by this technique (12). On the other hand, 987P pili from some strains tend to aggregate and to be readily demonstrated in such preparations (8, 12). Material B may have contained pili which were not demonstrable by the methods used here.

In conclusion, we were unable to demonstrate pili in material B; however, material B probably contains an antigen which mediates the MRHA and adhesive activities of $3P^-$ ETEC.

TABLE 4. Effects of erythrocyte source and mannose on hemagglutination caused by purified materials A and B from *E. coli* VAC-1676

Purified material (1 mg of protein per ml)	D-Mannose concn (%)	Hemagglutination[a] of erythrocytes from:						
		Pigs	Sheep	Rabbits	Chickens	Guinea pigs	Cattle	Horses
A[b]	0	1+	0	2+	3+	4+	0	0
	0.5	0	0	2+	1+	1+	0	0
B	0	4+	4+	4+	4+	4+	0	1+
	0.5	4+	4+	4+	4+	4+	0	1+

[a] Hemagglutination evaluated in a range from 1+ (weak or questionable hemagglutination) through 4+ (instantaneous and complete hemagglutination).

[b] Contains pili demonstrable by negative stain and electron microscopy.

ACKNOWLEDGMENTS

This work was conducted with the technical assistance of S. M. Skartvedt and K. O. Schlueter. We are grateful to Fritz and Ida Ørskov for serotyping the $3P^-$ ETEC and to Doyle J. Evans for testing them for CFA/I and II.

LITERATURE CITED

1. **Bellamy, J. E. C., and S. D. Acres.** 1979. Enterotoxigenic colibacillosis in colostrum-fed calves: pathologic changes. Am. J. Vet. Res. **40**:1391–1397.
2. **Burrows, M. R., R. Sellwood, and R. A. Gibbons.** 1976. Haemagglutinating and adhesive properties associated with the K99 antigen of bovine strains of *Escherichia coli*. J. Gen. Microbiol. **96**:269–275.
3. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis.
4. **Evans, D. J., Jr., D. G. Evans, and H. L. DuPont.** 1979. Hemagglutination patterns of enterotoxigenic and enteropathogenic *Escherichia coli* determined with human, bovine, chicken, and guinea pig erythrocytes in the presence and absence of mannose. Infect. Immun. **23**:336–346.
5. **Guinée, P. A. M., J. Veldkamp, and W. H. Jansen.** 1977. Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*. Infect. Immun. **15**:676–678.
6. **Isaacson, R. E.** 1977. K99 surface antigen of *Escherichia coli*: purification and partial characterization. Infect. Immun. **15**:272–279.
7. **Isaacson, R. E., P. C. Fusco, C. C. Brinton, and H. W. Moon.** 1978. In vitro adhesion of *Escherichia coli* to porcine small intestinal epithelial cells: pili as adhesive factors. Infect. Immun. **21**:392–397.
8. **Isaacson, R. E., B. Nagy, and H. W. Moon.** 1977. Colonization of porcine small intestine by *Escherichia coli*: colonization and adhesion factors of pig enteropathogens that lack K88. J. Infect. Dis. **135**:531–539.
9. **Levine, M. M., M. B. Rennels, V. Daya, and T. P. Hughes.** 1980. Hemagglutination and colonization factors in enterotoxigenic and enteropathogenic *Escherichia coli* that

cause diarrhea. J. Infect. Dis. **141**:733–737.

10. **Moon, H. W.** 1978. Pili as protective antigens in vaccines for the control of enterotoxigenic *Escherichia coli* infections, p. 393–410. *In* S. D. Acres (ed.), Proceedings of the 2nd Symposium on Neonatal Diarrhea. Veterinary Infectious Disease Organization, University of Saskatchewan, Saskatoon.
11. **Moon, H. W., E. M. Kohler, R. A. Schneider, and S. C. Whipp.** 1980. Prevalence of pilus antigens, enterotoxin types, and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. Infect. Immun. **27**:222–230.
12. **Moon, H. W., B. Nagy, and R. E. Isaacson.** 1977. Intestinal colonization and adhesion by enterotoxigenic *Escherichia coli*: ultrastructural observations on adherence to ileal epithelium of the pig. J. Infect. Dis. **136**:S124–S129.
13. **Nagy, B., H. W. Moon, and R. E. Isaacson.** 1976. Colonization of porcine small intestine by *Escherichia coli*: ileal colonization and adhesion by pig enteropathogens that lack K88 antigen and by some acapsular mutants. Infect. Immun. **13**:1214–1220.
14. **Runnels, P. L., H. W. Moon, and R. A. Schneider.** 1980. Development of resistance with host age to adhesion of K99$^+$ *Escherichia coli* to isolated intestinal epithelial cells. Infect. Immun. **28**:298–300.
15. **Smith, H. W., and M. B. Huggins.** 1978. The influence of plasmid-determined and other characteristics of enteropathogenic *Escherichia coli* on their ability to proliferate in the alimentary tracts of piglets, calves, and lambs. J. Med. Microbiol. **11**:471–492.
16. **Sojka, W. J., C. Wray, and J. B. Morris.** 1978. Passive protection of lambs against experimental enteric colibacillosis by colostral transfer of antibodies from K99-vaccinated ewes. J. Med. Microbiol. **11**:493–499.
17. **Stirm, S., F. Ørskov, I. Ørskov, and A. Birch-Andersen.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. III. Morphology. J. Bacteriol. **93**:740–748.
18. **Swaney, L. M., Y. P Liu, C. M. To, C. C. To, K. Ippen-Ihler, and C. C. Brinton, Jr.** 1977. Isolation and characterization of *Escherichia coli* phase variants and mutants deficient in type 1 pilus production. J. Bacteriol. **130**:495–505.