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Age-Specific Colonization of Porcine Intestinal Epithelium by 987P-Piliated Enterotoxigenic *Escherichia coli*

EVELYN A. DEAN,* SHANNON C. WHIPP, AND HARLEY W. MOON

National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

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Neonatal (<1-day-old), 3- and 7-day old, and older (3-week-old postweaning) pigs were challenged by intragastric inoculation with 987P-piliated (987P$^+$) enterotoxigenic *Escherichia coli* (ETEC) 987. Neonatal pigs were colonized (i.e., there were $\geq 10^8$ CFU of test strain per 10-cm ileal segment) and developed diarrhea. Intestinal colonization and the incidence and severity of diarrhea were lower in 3- and 7-day old pigs than in neonates. Older pigs were not colonized and did not develop diarrhea following oral inoculation with five strains of 987P$^+$ ETEC. Strain 987 (987P$^+$) adhered in vitro to intestinal epithelial cell brush borders isolated from both neonatal (sensitive) and older (resistant) pigs. The in vivo growth and expression of 987P pilus by strain 987 in ligated ileal loops created in neonatal and older pigs were similar. The in vivo adherence of 987P$^+$ ETEC to intestinal epithelium in ligated ileal loops in neonatal and older pigs was compared. In neonatal pigs, most of the bacteria were in layers associated with the villous epithelium. In older pigs, most of the bacteria were associated with mucuslike material in the intestinal lumen. We concluded that swine develop an innate resistance to 987P$^+$ ETEC by 3 weeks of age. This resistance does not appear to be due to an absence of 987P-specific receptors in the intestines of the older pig or to an inability of 987P$^+$ bacteria to grow and express pili in the older pig. We hypothesized that the resistance of older pigs to 987P-mediated disease is due to release of 987P-specific receptors into the intestinal lumen, where these receptors facilitate bacterial clearance rather than bacterial adherence to intestinal epithelium and colonization.

The ability to colonize the small intestine is an important virulence attribute of enterotoxigenic *Escherichia coli* (ETEC). 987P, K99, and K88 pili (fimbriae) are proteinaceous, filamentous appendages on the bacterial surface that promote the adherence of ETEC to the epithelium of the small intestine of neonatal pigs and thus facilitate intestinal colonization and diarrhea. Cell surface characteristics of the host also play an important role in the pathogenesis of ETEC diarrhea; i.e., pilus-specific receptors must be available on intestinal epithelium for pilus-mediated adherence (7, 10). The role of pilus-specific receptors in the pathogenesis of ETEC diarrhea was best demonstrated by the identification of pigs that are genetically resistant to K88-mediated adhesion and hence to colonization and diarrhea caused by K88-piliated ETEC. The small-intestine mucosae of K88-resistant pigs lack the receptor for the K88 pilus (11, 20). To date, no pigs lacking receptors for either the K99 or 987P pilus have been identified, and piglets that lack receptors for K88 are fully susceptible to 987P$^+$ and K99$^+$ ETEC (21).

ETEC cause diarrhea in swine during the immediate neonatal period (<6 days of age) and immediately after weaning (3 to 9 weeks old). Strains of ETEC that produce 987P or K99 pili are commonly associated with diarrhea in neonatal pigs but are only infrequently associated with disease in postweaning (older) pigs (22, 23). In contrast, ETEC that produce K88 pili are commonly associated with diarrhea in pigs of both ages (23). With the development of a reproducible model of postweaning diarrhea in pigs (19), it is now possible to determine the role of pili in the pathogenesis of diarrhea in postweaning pigs.

It is not known whether older pigs are susceptible to colonization and disease following experimental inoculation with 987P$^+$ ETEC. The mechanisms of age-related resistance to 987P-mediated ETEC disease are not known. The objectives of the experiments described in this manuscript were to determine (i) whether 987P$^+$ ETEC can cause diarrhea in older pigs; (ii) whether 987P receptors are present in older pigs; and (iii) whether 987P$^+$ bacteria can grow, express 987P pili, and adhere in vivo in older pigs. We hypothesize that resistance of older pigs to 987P-mediated disease results from increased release of 987P-specific receptors into the intestinal lumen, where these receptors facilitate bacterial clearance rather than bacterial adherence to epithelium and colonization.

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MATERIALS AND METHODS

Bacteria. The *E. coli* strains used are described in Table 1. Unless otherwise noted, cultures were grown and maintained on tryptic soy agar plates under conditions that promoted 987P expression (9). Cells from isolated colonies were tested for 987P pilus expression by slide agglutination with specific anti-987P serum (17), inoculated into tryptic soy broth, and incubated at 37°C for 18 h without shaking. Stock inocula, containing 10^9 to 10^{10} CFU/ml, were prepared as previously described (14), stored with 20% glycerol at −80°C, and diluted immediately prior to use. All cultures were tested for the presence of 987P pili by slide agglutination before use as inocula. The percentage of 987P-piliated bacteria (determined for strains 987 and I36 only) in the diluted inocula was estimated by colony blot immunoassay (see below). The number of viable cells in the diluted inocula was estimated by plate counts (16).

Anti-987P pilus serum. The preparation of rabbit anti-987P pilus serum was described previously (17). The immunoglobulin G fraction was prepared by protein A affinity chromatography with Affipak prepacked columns and MonoPure

* Corresponding author.

TABLE 1. *E. coli* strains used

Strain[a]	Serotype	Enterotoxin genes[b]	987P antigen[c]
987	O9:K103:NM	STb, STp	+
I36	O9:K103:NM	STb, STp	−
74-5208	O20:K101:NM	STb, STp	+
1635	O141:NM	STb, STp	+
1636	O8:K85:NM	STb, STp	+
1661	O141:K.:NM	STb, STp	+
1708	O141:K.:NM	None	−
1712	Unidentified	None	−

[a] All strains were originally isolated from pigs with diarrhea, except strain I36, which was derived from strain 987 (8).

[b] The presence of enterotoxin genes was determined by using DNA probes and the DNA colony hybridization technique (15). STp, Porcine heat-stable enterotoxin a; STb, heat-stable enterotoxin b; none, negative with STp, STb, and heat-labile enterotoxin probes.

[c] The in vivo expression of 987P antigen was determined by IFA with anti-987P serum.

buffers (Pierce Chemical Co., Rockford, Ill.) as specified by the manufacturer.

Animals. Twenty-eight neonatal piglets (<1 day old, colostrum deprived, and unfed) were obtained by cesarean section from seven crossbred swine from a single herd (H1). We also used 16 3-day-old, 8 7-day-old, and 71 older (3-week-old, 1 day postweaning) pigs which had been naturally farrowed at the National Animal Disease Center by 14 crossbred swine (gilts) from three herds (H1, H2, and H3). Weaned pigs were fed a postweaning diet without oxytetracycline, as described previously (19).

In vitro adhesion assays. In vitro adhesion assays with *E. coli* 987 and I36 and isolated small-intestine epithelial-cell brush borders were performed as previously described (3, 20).

Inoculations. Neonatal pigs (<1 day old) were inoculated intragastrically (16) with 12 ml of tryptic soy broth containing 0.7×10^{10} to 1.0×10^{10} CFU. Inoculated animals were kept (not fed) in individual cardboard boxes at 37°C for 6 or 18 h postinoculation. Intragastric inoculation of 3- and 7-day-old pigs was the same as that of neonates; however, inoculated pigs were left with the dam for 6 h to 6 days postinoculation.

Older pigs were inoculated intragastrically under conditions that consistently produced diarrhea with K88-piliated *E. coli*, as previously described (19). Briefly, pigs were weaned 1 day prior to challenge and inoculated via stomach tube with 0.4×10^{10} to 1.1×10^{10} bacteria in 60 ml of half-strength tryptic soy broth containing 1.2% sodium bicarbonate. The pigs were housed with similarly challenged pigs, two to four pigs per room, for 6 h to 6 days postinoculation.

At the times noted, animals were sacrificed, and sections of ileum were taken for counts of viable bacteria and for immunofluorescence and morphologic studies, as previously described (16, 19). Pigs having $\geq 10^8$ CFU per segment (10 cm for neonatal and 5 cm for older pigs) were considered colonized. In vivo expression of 987P antigen was determined by the indirect immunofluorescence assay (IFA) with anti-987P serum (16).

Ligated intestinal loops. Ligated ileal loops (10 cm) were created in neonatal or older pigs, as described elsewhere (1), and inoculated with 0.7×10^8 to 1.1×10^8 CFU of test strain per loop. Animals were necropsied 6 h postinoculation, and viable bacterial counts were determined (16). Bacterial growth was expressed as the increase in $\log_{10}$ CFU per loop. 987P antigen was determined by IFA, as described above, and by colony blot immunoassay, as described below.

Association index. The distribution of the test *E. coli* bacteria in ligated ileal loops was morphologically determined by low-power microscopic examination of frozen sections stained with anti-O9:K103 serum conjugated to fluorescein isothiocyanate and by high-power examination of sections stained with toluidine blue (1, 16). The association index was obtained by multiplying the values obtained in evaluating the extent and intensity of bacterial fluorescence (from 1 for no fluorescence to 5 for maximal fluorescence) and the contiguity of bacteria to the epithelial cells (from 1 for no contiguity to 5 for maximal contiguity) as previously described (1, 16).

Colony blot immunoassay for 987P antigen. The expression of 987P pili by bacterial cells in the inocula and by test strain isolates from ligated loops was estimated by a colony blot immunoassay. Isolated colonies from tryptic soy agar plates were blotted onto nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.), and 987P antigen was detected by an indirect immunoperoxidase procedure with rabbit anti-987P immunoglobulin G and horseradish peroxidase-conjugated protein A (Sigma Chemical Co., St. Louis, Mo.). Additional binding sites on the filters were blocked by three washes (10 min) in 0.05% Tween in phosphate-buffered saline as described by DeBlas and Cherwinski (6). The filters were sequentially incubated (1 h) with rabbit anti-987P immunoglobulin G (2.5 μg/ml), in horseradish peroxidase-conjugated protein A (125 ng/ml), and with substrate (0.5 mg of 4-chloro-1-naphthol per ml, 0.015% H_2O_2, and 16.7% methanol in phosphate-buffered saline). Between each step, the filters were given three 10-min washes with 0.05% Tween 20 in phosphate-buffered saline, except for the final three washes (before incubation with the substrate), when the Tween was omitted. All steps were performed at ambient temperature. $987P^+$ colonies appeared as purple (peroxidase-active) spots; $987P^-$ colonies remained colorless. Results were expressed as percent 987P piliation [(number of $987P^+$ colonies/number of colonies tested) × 100%]. Strains 987 ($987P^+$) and I36 (a P^- variant derived from strain 987) served as positive and negative controls, respectively.

TABLE 2. Incidence of diarrhea and intestinal colonization in pigs challenged by intragastric inoculation with $987P^+$ *E. coli*

Inoculum	Age	No. of litters	No. of pigs with diarrhea/no. tested[a]		No. of pigs colonized/no. tested[b]	
			6 h	24 h	6 h	24 h
987	<1 day	3	3/4	2/2	4/4	2/2
987	3 days	2	ND[c]	2/10	ND	2/4
987	7 days	1	ND	3/8	ND	1/1
987	3 wk	7	0/3	0/21	0/3	0/3
$987P^+$ ETEC pool[d]	3 wk	3	ND	0/11	ND	0/1

[a] Incidence of diarrhea was noted at the indicated times postinoculation with *E. coli* 987; with <1-day-old pigs the second observation time was at 18 h rather than at 24 h.

[b] Colonization, defined as $\geq 10^8$ CFU of *E. coli* 987 per 10-cm (for pigs <1, 3, or 7 days old) or 5-cm (for 3-week-old pigs) ileal segment, was determined by plate counts performed at the indicated times postinoculation; with <1-day-old pigs, the second sampling time was at 18 h, as noted in footnote *a*.

[c] ND, Not determined.

[d] Pool contained 10^{10} CFU each of strains 74-5208, 1635, 1636, 1661, 1708, and 1712 (see Table 1).

TABLE 3. In vivo growth, 987P pilus expression, and epithelial association of *E. coli* 987 (987P$^+$) and I36 (987P$^-$) in ligated intestinal loops of neonatal and older pigs

Age of pig	Inoculum strain	No. of pigs[a]	Growth ($\Delta\log_{10}$)[b]	Expression of 987P antigen[c]	Association index[d]
<1 day	987	5 (a)	1.0 ± 0.1	5	ND[e]
		6 (b)	1.2 ± 0.3	6	8.8
<1 day	I36	6 (b)	1.2 ± 0.1	0	1.2
3 wk	987	3 (c)	0.9 ± 0.1	3	2.4
		3 (d)	1.0 ± 0.3	3	2.2
3 wk	I36	3 (c)	1.4 ± 0.2	1	3.4
		3 (d)	0.6 ± 0.1	1	2.1

[a] Letters in parentheses designate litter (see text).
[b] Growth is expressed as the increase in $\log_{10}$ CFU of the test strain per loop 6 h postinoculation ($\log_{10}$ final CFU per loop − $\log_{10}$ inoculum). Results are given as mean ± standard deviation.
[c] In vivo expression of 987P antigen was determined by IFA as described previously (16). Results are given as the number of loops with 987P$^+$ bacteria.
[d] The degree of bacterial adherence was determined as previously reported (1). 1, No villus-associated bacteria seen; 25, maximal; bacteria along the entire length of all villi.
[e] ND, Not determined.

RESULTS

Intragastric inoculations. To determine whether there are age-related effects on the susceptibility of pigs to 987P$^+$ ETEC, we challenged neonatal (<1-day-old), 3- and 7-day-old, and 3-week-old (older) pigs by intragastric inoculation with *E. coli* 987 (987P$^+$). The results are shown in Table 2. Strain 987 was highly virulent in neonatal pigs, as has been reported previously (8, 16); five of six pigs developed severe diarrhea, and six of six were colonized 6 or 18 h after intragastric inoculation with strain 987. However, the incidence and severity of diarrhea in both 3- and 7-day-old pigs following similar challenge were lower than in the <1-day-old pigs; diarrhea occurred in 2 of 10 and 3 of 8 pigs, respectively. The small intestines of two diarrheic pigs (one 3 days old and one 7 days old) were examined 24 h postinoculation for bacteria. Both were judged to be colonized (>10^8 CFU/10 cm). One of three nondiarrheic 3-day-old pigs examined 24 h postinoculation was also colonized. The remaining pigs with diarrhea (one 3 days old and two 7 days old) recovered by day 4 postinoculation. In contrast, *E. coli* 987 did not produce diarrhea in any of the 3-week-old pigs, and intestinal colonization was not observed in any of the pigs of this age examined. Likewise, none of 11 older pigs challenged with a pool containing a mixture of four other 987P$^+$ ETEC strains (fully virulent in neonatal pigs; results not shown) and two nonenterotoxigenic *E. coli* strains (Table 1) developed diarrhea; the single pig examined 24 h postinoculation was not colonized.

In vitro adherence of *E. coli* 987. *E. coli* 987 adhered in vitro to brush borders isolated from small-intestine epithelial cells of older pigs. The in vitro adhesion to brush borders from older pigs showed a similar distribution, and the number of adherent bacteria (10 to 30 bacteria per brush border) was similar to what was seen with neonatal pigs (7). Brush borders were obtained from 9 of the 24 older pigs (Table 2) that did not develop diarrhea following challenge with strain 987, from two unchallenged 4-week-old postweaning pigs, and from 4-week-old pigs used to develop the K88 model of postweaning diarrhea (19). 987P$^+$ bacteria adhered to brush borders lacking the K88 receptor, as well as to those containing the K88 receptor. Strain I36 (987P$^-$) did not adhere to brush borders from any of the pigs.

Growth, 987P pilus expression, and adherence of *E. coli* 987 in ligated loops. The growth of piliated *E. coli* 987 in ligated ileal loops was similar in neonatal and older pigs (Table 3). At 6 h postinoculation, the total number of CFU of test strain per loop was approximately 10-fold greater than that of the inoculum. The growth of strains 987 and I36 was similar in pigs of both ages. Some litter-to-litter variation was seen.

987P pili undergo phase variation (2, 17), and this variation is affected by bacterial growth conditions (9). The expression of 987P pili by bacteria grown in ligated ileal loops was monitored to determine whether growth conditions in the small intestines of older pigs inhibited 987P expression. 987P antigen was detected by IFA in ligated ileal loops in all neonatal and older pigs inoculated with strain 987 (987P$^+$); 987P antigen was also detected in loops inoculated with strain I36 in two of the older pigs (Table 3). 987P$^+$ colonies were also identified by the colony blot immunoassay among bacterial isolates from all loops (11 of 11 neonatal pigs and 6 of 6 older pigs) 6 h after inoculation with strain 987. However, there was considerable pig-to-pig and litter-to-litter variation in the percentage of 987P$^+$ isolates from ligated loops (Fig. 1). In one of the two litters of neonatal pigs (litter b), the percentage of 987P$^+$ loop isolates (56% ± 14% [mean ± standard deviation]) was higher than that in the inoculum (40%). However, in the other neonatal litter (25% ± 11%) and in both older litters (19% ± 12% and 14% ± 17%), the percentage of 987P$^+$ loop isolates was lower than that in the inoculum (41% ± 2%). A low percentage (<1%) of I36 isolates from ligated ileal loops in four of six neonatal pigs and two of six older pigs inoculated with strain I36 were 987P piliated; however, no piliated colonies could be detected in any of the I36 inocula by colony blot immunoassay. When strain 987 was grown under conditions that suppressed 987P pilus expression in vitro, no piliated colonies were detected by colony blot immunoassay. However, in vivo 987P antigen expression was demonstrated by both IFA

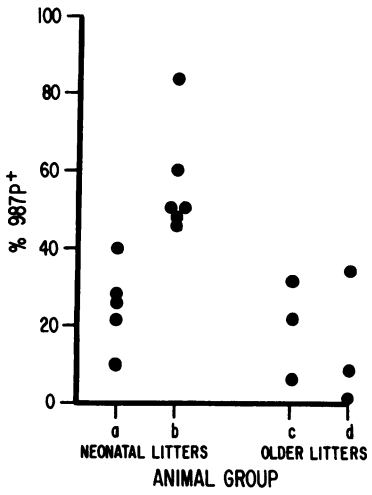

FIG. 1. Percentage of 987P$^+$ *E. coli* isolated from ligated intestinal loops of neonatal (<1-day-old) and older (3-week-old, 1 day postweaning) pigs. The percentage of 987P$^+$ bacteria isolated from ligated intestinal loops 6 h postinoculation with strain 987 was estimated by colony blot immunoassay as described in Materials and Methods. The inoculum contained 41 ± 2% 987P$^+$ bacteria, as estimated by this assay. Results are expressed as percent 987P$^+$ [(number of 987P$^+$ colonies/total number of colonies tested) × 100%]; 50 to 450 colonies per loop were tested. Two litters each of neonatal (a and b) and older (c and d) pigs were tested. Each dot represents results from a single pig.

and colony blot immunoassay in loops in all neonatal and older pigs inoculated with nonpiliated inocula (24% ± 13% and 9% ± 8% of isolates from ligated ileal loops of neonatal and older pigs, respectively, were positive for 987P antigen by colony blot).

The question of whether 987P$^+$ bacteria adhered to intestinal epithelium of older pigs in vivo was also addressed by using ligated loops. The use of loops enabled bacterial numbers to be maintained above 10^6 so that adherence (bacterial layers) and association to intestinal epithelia could be monitored by IFA. Also, effects of age-related differences in intestinal motility on bacterial adherence were reduced in ligated loops. Most of the bacteria in loops in neonatal pigs inoculated with strain 987 were in bacterial layers associated with the villous epithelium (Fig. 2a); strain I36 did not form bacterial layers. In contrast, most of the strain 987 or I36 bacteria in loops in older pigs were disbursed in mucus and cellular debris in the central lumen (Fig. 2b and c), with individual cells occasionally associated with the mucosa. In these cases, higher-power observation showed that these cells were associated with a thin mucuslike layer covering the mucosa and were not in direct contact with the brush borders. The epithelium-associated bacterial layers in neonates were more extensive and contained more bacteria than those in the older pigs. These differences in the extent and number of bacteria in layers resulted in a higher association index in the neonates (Table 3).

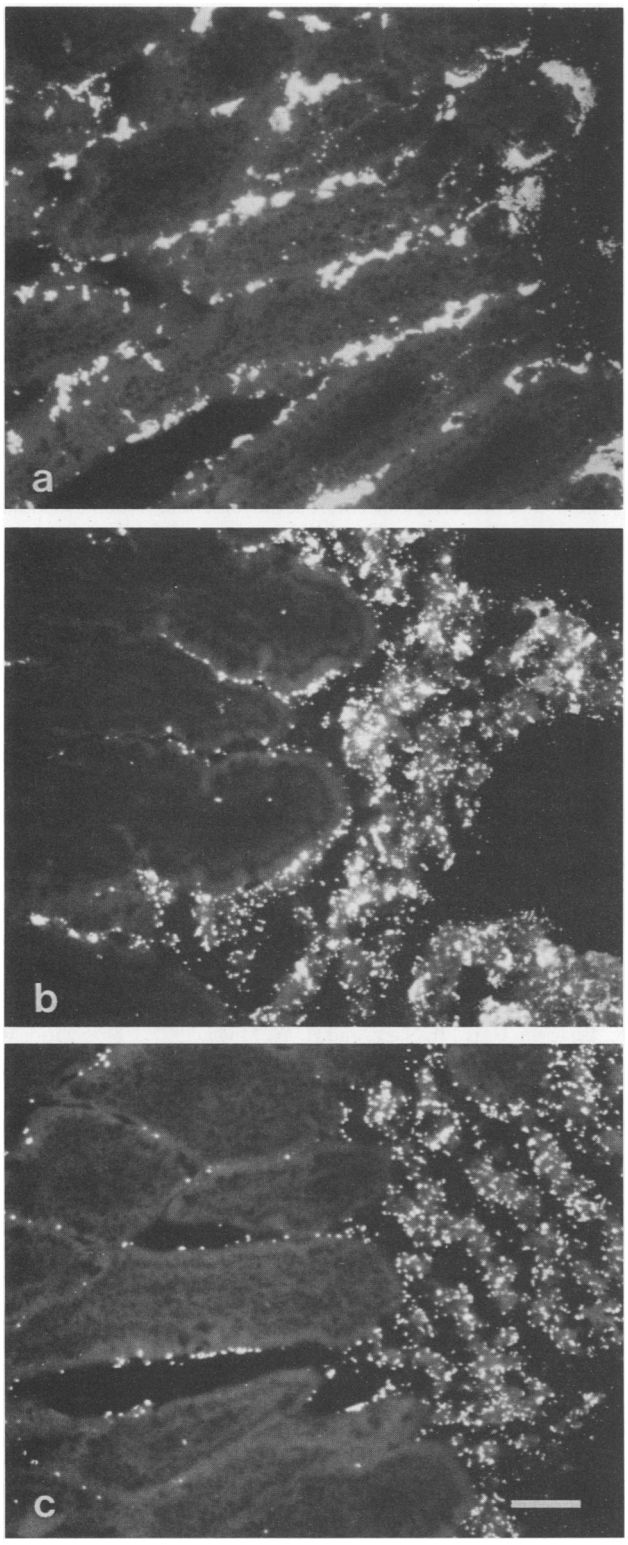

FIG. 2. Association of strain 987 *E. coli* with porcine small-intestine epithelium. Frozen sections of ileal loops from neonatal (a) and older (b and c) pigs 6 h after challenge with *E. coli* 987 (a and b) or I36 (c) are shown. Sections were stained with fluorescein-conjugated anti-O9:K103 serum (bar = 20 μm). In the neonate, most of the strain 987 bacteria are in layers adherent to villi (panel a). However, in the older pig, most of them are in mucus in the intestinal lumen (panel b). The distribution of piliated (strain 987) and nonpiliated (strain I36) bacteria in older pigs is similar (compare panels b and c).

DISCUSSION

Strains of ETEC that produce 987P pili colonized the small intestines and caused diarrhea in neonatal pigs but not in older pigs. We have considered several possible explanations for the age specificity of colonization by 987P$^+$ ETEC in pigs: (i) there are no available intestinal receptors for the 987P pilus in the older pig; (ii) there are quantitative differences in the growth of 987P$^+$ *E. coli* in neonatal and older pigs (less growth in older pigs); (iii) there are effects of increased intestinal motility on 987P-mediated adherence; (iv) there are quantitative differences in the expression of 987P pili by bacteria in neonates and older pigs (less expression in older pigs); (v) there are blocking substances such as excess receptor (blocking), pilus or receptor analogs, or antibodies that impair pilus-receptor interactions in the small intestines of older pigs; and (vi) there are quantitative or qualitative differences in epithelial receptors for the 987P pilus that develop with age.

The ability of 987P$^+$ *E. coli* to adhere in vitro to intestinal epithelial cell brush borders isolated from both older pigs and neonatal pigs indicates that there are receptors for the 987P pilus on brush borders of both older and neonatal pigs and that age-related resistance to 987P-mediated disease is apparently not associated with an absence of 987P receptors in older pigs. In contrast to 987P$^+$ ETEC, age-related resistance to K99-mediated disease is associated with an age-dependent decrease (or masking) of intestinal receptors for K99 pili, since K99$^+$ bacteria do not adhere to epithelial cells of older (resistant) pigs either in vivo or in vitro (13, 18). K88$^+$ ETEC cause disease in both neonatal and older K88-susceptible pigs and adhere both in vivo and in vitro to intestinal epithelia of K88-susceptible pigs of both ages, but not to those of any K88-resistant pigs (19, 20).

Resistance of older pigs to 987P$^+$ ETEC-mediated disease does not appear to be due to age-related effects on bacterial

growth or 987P pilus expression. We found no differences in the growth of strain 987 (987P$^+$) in ligated loops in neonatal and older pigs (Table 2). 987P pilus antigen was detected, by both IFA and colony blot immunoassay, on bacteria from all ligated loops in neonatal and older pigs inoculated with *E. coli* 987, even when the inoculum was prepared under conditions that inhibited 987P expression. Furthermore, our observation that strain I36 reverted to the 987P$^+$ phase when grown in the small intestines of both neonatal and older pigs indicated that in vivo growth conditions in pigs of both ages are favorable to 987P pilus expression. The ability of strain I36, a 987P$^-$ phase variant that is stable in vitro (8), to revert to 987P$^+$ phase in vivo in neonatal pig ileal loops has previously been described (12).

Strain 987 bacteria did not adhere to the small-intestine epithelium in ligated ileal loops created in older pigs, even though bacterial numbers were kept high (1) and intestinal-motility effects were minimized. The distribution of strain 987 (987P$^+$) bacteria in the older pig was similar to that of strain I36 (987P$^-$) (Table 3; Fig. 2). The association of 987P$^+$ bacteria with intestinal mucus and cellular debris in the older pigs (association index, 2.3), rather than with intestinal epithelium as in the neonatal pigs (association index, 8.8), is consistent with quantitative or qualitative age-related differences in the 987P receptors or with altered receptor distribution in older pigs.

The possibility that blocking substances (987P-specific antibodies or pilus or receptor analogs) were present in older pigs was not directly addressed. However, we observed that 987P-specific antibody titers in milk at the time of weaning were low ($\leq$8; results not shown). Furthermore, older pigs were weaned 24 h before challenge to minimize any possible effects of maternal 987P-specific antibodies on intestinal colonization by 987P$^+$ *E. coli*. Since 3- and 7-day-old pigs were not weaned before or after challenge, maternal 987P-specific antibody may have contributed to their increased resistance to 987P-mediated intestinal colonization and diarrhea production. 987P-specific antibodies are an unlikely explanation for age-related resistance to 987P-mediated disease, since K88$^+$ ETEC are at least as common as 987P$^+$ ETEC in the swine population and K88$^+$ ETEC-mediated disease commonly occurs in older pigs (22). Furthermore, older pigs in these herds that were not susceptible to infection with 987P$^+$ ETEC were susceptible to infection with K88$^+$ ETEC when raised and weaned in this fashion (19). Although these observations are consistent with the conclusion that antibodies are not responsible for the age-related resistance of swine to 987P-piliated ETEC, the involvement of antibodies in this resistance has not yet been convincingly ruled out.

The lack of correlation between in vitro adherence and in vivo intestinal colonization and diarrhea production by 987P$^+$ ETEC in older pigs is similar to what occurs in adult rabbits. A receptor for the 987P pilus has been isolated from rabbits (3, 4), and antibody against this rabbit 987P receptor binds to brush borders isolated from older pigs (E. A. Dean, unpublished observation). Therefore, we hypothesize that the intestinal epithelium of older pigs contains a receptor for the 987P pilus that is similar to the rabbit receptor. Preliminary studies indicate that brush borders from older pigs contain a 987P pilus-binding component that comigrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the purified rabbit receptor for the 987P pilus (4). Antibodies against the rabbit 987P receptor also bind to the small-intestine epithelium of neonatal pigs (5). The receptor for the 987P pilus in neonatal pigs has not yet been identified.

We hypothesize that the 987P receptors in the small intestines of neonatal and older pigs are similar and that the receptor in older pigs is more easily released from the intestinal epithelium. The ability of antibodies against the rabbit 987P pilus receptor to bind to both neonatal- and older-pig intestinal epithelium is consistent with this hypothesis. Released 987P receptors may have a greater affinity for 987P, occur in greater quantity, or establish earlier contact with 987P$^+$ piliated bacteria than membrane-bound 987P receptors. Thus, adherence of 987P-piliated bacteria to an easily released receptor entrapped in luminal mucus might facilitate bacterial clearance of 987P$^+$ ETEC, similar to the clearance of nonpiliated strain I36. The association of 987P$^+$ bacteria with intestinal mucus and epithelial cell debris in ligated loops in older pigs, in contrast to their association with intestinal epithelium in neonates, is consistent with this hypothesis.

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