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INTESTINAL COLONIZATION BY ENTEROTOXIGENIC 'ESCHERICHIA COLI'.(U)
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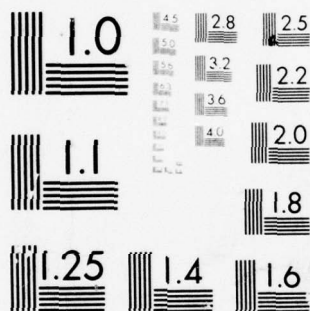
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Escherichia coli

ANNUAL REPORT

HARLEY W. MOON

December, 1977

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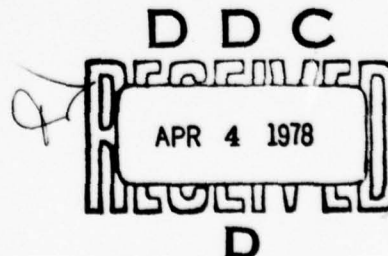
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20315

Contract No. DAMD 17-75-C-5014

National Animal Disease Center
U.S. Department of Agriculture, and
Department of Pathology, Iowa State University
Ames, Iowa 50010

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Summary

and
This study was designed to test the hypothesis that: (1) enterotoxigenic E. coli characteristically colonize mammalian small intestine by adhering to the epithelial surface; (2) that adhesion, referred to above and involving strains lacking K88 antigen, occurs between pili on the bacterial surface and microvilli on villous absorptive cells of the host. *This document contains two closely related papers, including*

1. Immunization Against Enterotoxigenic Escherichia coli by Vaccinating with Purified Pili

A. Pregnant swine (gilts) were vaccinated parenterally with a purified pilus preparation from porcine enterotoxigenic Escherichia coli strain 987 (09:K103:NM). Gilts injected with placebo served as controls. Suckling pigs born to gilts in both groups were challenged intragastrically with virulent strain 987. The percentage of deaths, incidence and duration of diarrhea, numbers of E. coli in ileum, and E. coli attachment to villous epithelium all were less in pigs suckling vaccinated gilts than in those suckling the controls. These results are consistent with the hypothesis that pili of some enterotoxigenic E. coli act as virulence attributes by facilitating adhesion to intestinal epithelium.

This work is presented in detail in the manuscript in the Body of the report, "Immunization of Suckling Pigs Against Enterotoxigenic Escherichia coli Infection by Vaccinating Dams with Purified Pili," *cont on p. 1473A* which has been submitted for publication in Infection and Immunity.

B. The data presented here demonstrate that vaccination of pregnant gilts with purified pili provides passive protection of their suckling pigs against diarrheal disease caused by an ETEC strain possessing the homologous pilus. Pigs born to gilts that had been vaccinated with K99 had a lower incidence of death, decreased incidence and duration of diarrhea, and better weight gain than pigs born to 987P or control vaccinated gilts when challenged with the K99 strain 431. Similarly, pigs born to 987P vaccinated gilts when challenged with either strains 987 or 74-4208 had lower incidences of death and equal or lower incidences and durations of diarrhea than their K99 vaccinated counterparts. In no instance did a pilus confer protection upon pigs challenged with a totally heterologous strain. Thus, for example, vaccination with K99 did not protect against diarrheal disease caused by strain 987.

This work is presented in detail in the manuscript in the Body of the report, "Immunization Against Enterotoxigenic Escherichia coli Infection by Vaccination with Purified Pili," which is in press, Proc. 13th Joint Conference on Cholera, U.S.-Japan Cooperative Medical Science Program, Atlanta, Ga., Sept. 19-21, 1977.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences - National Research Council.

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Immunization of Suckling Pigs
Against Enteric Enterotoxigenic Escherichia coli Infection
by Vaccinating Dams with Purified Pili

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Running title: E. coli pilus vaccine

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ABSTRACT

Pregnant swine (gilts) were vaccinated parenterally with a purified pilus preparation from porcine enterotoxigenic Escherichia coli strain 987 (09:K103:NM). Gilts injected with placebo served as controls. Suckling pigs born to gilts in both groups were challenged intragastrically with virulent strain 987. The percentage of deaths, incidence and duration of diarrhea, numbers of E. coli in ileum, and E. coli attachment to villous epithelium all were less in pigs suckling vaccinated gilts than in those suckling the controls. These results are consistent with the hypothesis that pili of some enterotoxigenic E. coli act as virulence attributes by facilitating adhesion to intestinal epithelium.

MATERIALS AND METHODS

Swine. Seventeen gilts were bred when 7.5 months old. These and their offspring were the experimental animals.

E. coli strain. Strain 987 (09:K103:NM) was isolated from the small intestine of a baby pig with enteric colibacillosis. This strain produces heat stable but not heat labile enterotoxin, is piliated, colonizes porcine ileum with adhesion to villous epithelium, and causes profuse diarrhea in newborn pigs (5,7,11,12).

Preparation of the vaccine and immunization. The preparation of purified pilus material from strain 987 will be reported separately (3) and basically was a modification of the procedure of Brinton (2). The purified pilus material was administered in phenolized (0.5%) saline. The placebo consisted of phenolized saline only. The gilts weighed 122-160 kg during the last trimester of pregnancy when 9 were injected with the pilus vaccine (vaccinated group) and 8 were injected with the placebo (control group). The vaccine contained 5 mg of protein in 10 ml of saline and was inoculated subcutaneously in the flank 21-27 and again 7-13 days before parturition except for the first two gilts, which received 8 mg of pilus protein at the first injection. The pilus vaccine and the placebo were coded so that people vaccinating the gilts and making clinical and laboratory observation on the pigs did not know which material contained the pili.

Blood samples were taken from all gilts immediately before each vaccination and immediately before farrowing. During parturition, the gilts were given 2 U.S.P. units of Oxytocin (P.O.P., Armour-Baldwin Laboratories) intramuscularly, and 100-200 ml of colostrum was manually drawn from each gilt. Casein was precipitated from colostrum samples with the addition of renin and calcium chloride in a concentration sufficient to create a firm clot at 42 C. Colostrum whey and serum samples were filtered through 0.22 μ m Millipore filter and stored at -70 C.

Challenge. At birth, pigs were separated from the gilts for 2-6 h, until all were born. They were then weighed and returned to the gilts for 30 min and allowed to suckle. After this initial colostrum intake, each pig was inoculated intragastrically with strain 987. The stock inoculum was kept frozen at -70 C, in 10% glycerol and contained 5.4×10^8 viable bacteria per ml. Immediately before the inoculation of each litter, one vial containing 1 ml of the frozen inocula was thawed out and 0.25 ml of it was diluted in 15 ml of cold trypticase soy broth (TSB). One ml of this TSB dilution, containing about 9×10^6 viable bacteria, was added to an additional 10 ml of cold TSB and inoculated into each pig intragastrically via stomach tube. This dose was chosen because preliminary experiments indicated it was an approximate LD_{50} .

Observations on pigs. Pigs were weighed individually, and their clinical status was recorded at challenge, at 16 h post challenge and again at 2, 3, 4, 5, and 6 days post challenge. One pig from each litter was selected (the one that had lost the most or gained the least weight) 16 h post challenge and killed. A 10 cm segment of ileum was removed, and the viable number and degree of adhesion (association index) of strain 987 were determined as described earlier (11) except that efforts were made to determine the association index even of those segments with $< 10^8$ E. coli. All pigs that died during the experiment were examined postmortem and only those with gross lesions compatible with enteric colibacillosis (9) were included in the results. Two pigs died of disease other than colibacillosis: one from a vaccinated gilt (died of starvation); the other from a nonvaccinated gilt (died of bleeding from the cord). Neither of these pigs had diarrhea.

Preparation of anti 987-pilus serum. This was described in detail earlier (12). Briefly, antisera against the piliated form of strain 987 were produced as described by Edwards and Ewing (6) for O:K antisera. Antibodies against O and K antigens were removed from the sera by absorption with a noncapsulated, nonpiliated and a capsulated, nonpiliated mutant of strain 987. The resulting antisera did not agglutinate the large, opaque colonies of strain 987 grown on 5% sheep blood agar plates; which were found by electron microscopy to be poorly piliated or nonpiliated. However, the antisera did agglutinate the small, transparent colonies which were found by electron microscopy to be richly piliated (P^{++}).

RESULTS

Intestinal colonization, adhesion of bacteria to intestinal villi, death, and diarrhea all were significantly less, whereas weight gain was significantly more in the pigs from vaccinated gilts than in the pigs from the nonvaccinated gilts (control) (Table 1).

Intestinal colonization. There were significantly fewer viable bacteria of strain 987 per 10 cm of ileum of pigs from the immunized group (10^6 - 10^9) than from the nonimmunized group (10^6 - 10^{11}). Also small, translucent, richly piliated (P^{++}) colonies of strain 987, agglutinable in the anti-pilus serum (12), were present in a high proportion (38-99% of the total) of the population recovered from the ileum of 3 pigs tested from the control group. In contrast, no such P^{++} colonies were found in ileal isolates from the 3 pigs of the vaccine group so tested.

Association index. This index was used to express the degree of adhesion of the challenge strain to the ileal epithelium. In fluorescent antibody stained ileal sections from pigs in the vaccine group, the challenge bacteria were either too few to identify or were predominantly in the luminal area without any tendency to adhere to the intestinal epithelium (Fig. 1). Thus, all pigs of this group had low association indices (Table 1).

In contrast, 4 out of 8 pigs from the control group had high association indices; that is, adherent bacteria of strain 987 covered

the intestinal villi as a layer (Fig. 2, Table 1). The difference in association indices between the two groups was significant ($P < .05$).

Death losses. None of the pigs in the vaccine group died of enteric colibacillosis, but 30% of the pigs in the control group died of colibacillosis during the first 6 days of life. Most deaths occurred on the 2nd or 3rd day.

Diarrhea. Sixteen hours post challenge, 56% of the pigs from the vaccine group had diarrhea, whereas 72% of the pigs from the control group had diarrhea (Fig. 3). From that time, the number of pigs with diarrhea sharply decreased in the vaccine group and all these pigs were normal by 5 days post challenge. In contrast, the number of pigs in the control group with diarrhea decreased only after the 3rd post challenge day and 28% of the surviving pigs still had diarrhea by day 6 (Table 1, Fig. 3). The most striking difference between vaccine and control groups developed at the 3rd day post challenge.

Weight gain. The mean growth rate to 6 days of the litters from the vaccine group was 6.7 g/h but was significantly less ($P < .05$), 3.6 g/h, for those in the control group which survived to 6 days.

Litter related resistance. There was significant ($P < .005$) litter to litter variation in death loss within the control group (Table 1). Four out of 8 litters (dams 3, 7, 14 and 17) lost a total of one pig only, but the other four litters lost 16 pigs. At 16 h post exposure, pigs from dams 3, 7, 14 and 17 also had lower numbers of E. coli in their ilea and lower association indices than pigs from the other litters in the control group (Table 1).

DISCUSSION

The differences between the vaccine and control groups in incidence of death and diarrhea, as well as in weight gain, indicate that vaccination of gilts with purified pilus, of E. coli 987, provided passive protection for their pigs against the challenge with the parent strain. The comparatively lower association indices and viable numbers of E. coli in ilea of pigs from the vaccine group demonstrate that strain 987 did not attach and attain large numbers in the small intestine of the protected pigs. One plausible explanation for the latter is that the protection was due to anti pilus, anti adhesive antibodies. Because pigs do not acquire passive immunity in utero, these anti pilus antibodies were probably transmitted to the pigs via colostrum of the vaccinated gilts. The high proportion of P^{++} (small, pilliated) colonies in the ileal isolates of control pigs in contrast to those from the vaccine pigs is also consistent with the above explanation.

The litters of some control gilts were also relatively resistant to strain 987. This resistance could be the consequence of naturally acquired antibodies against this strain (4) or some other form of resistance to colonization (15).

The data are consistent with the hypothesis that pili of strain 987 act as virulence factors by facilitating adhesion to intestinal epithelium. Rutter and Jones (13,14) demonstrated that gilts vaccinated with K88 pili also protect their suckling pigs against challenge with K88⁺ enterotoxigenic E. coli. Our data provide a second example for protection of pigs against enteric enterotoxigenic E. coli infection by vaccinating their dams with homologous purified pili. Vaccination of dams with pili appears to be a useful method for preventing diarrheal disease caused by enterotoxigenic E. coli in suckling neonates.

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TABLE 1. Response of pigs, suckling vaccinated^a and control gilts, to challenge with enterotoxigenic *E. coli* 987

Group	Dam No.	16 h after challenge		6 days after challenge		
		Log ₁₀ <u>E. coli</u> / 10 cm ileum	Association Index	Survivors		
				Death/ total	Diarrhea/ total	Weight gain gram/h
Vaccinated	1	8.6	1.0	0/8	0/8	7.8
	2	8.1	1.7	0/7	0/7	8.9
	5	8.4	1.0	0/11	0/11	8.6
	6	8.9	1.0	0/8	0/8	6.5
	10	6.0	1.0	0/11	0/11	4.5
	11	7.9	1.0	0/6	0/6	5.0
	12	6.0	1.0	0/3	0/3	4.4
	15	6.0	2.5	0/7	0/7	7.2
	16	6.0	2.0	0/8	0/8	7.3
		$\bar{X} = 7.3^b$	$\cdot\bar{X} = 1.4$	$\Sigma = 0/69^c$	$\Sigma = 0/69$	$\bar{X} = 6.7$
Control	3	8.7	1.0	0/7	0/7	8.8
	4	10.2	5.0	7/10	3/3	1.3
	7	9.8	1.0	1/6	3/5	6.3
	8	8.9	4.2	6/11	1/5	1.3
	9	10.8	5.0	1/3	2/2	0.3
	13	10.4	4.2	2/8	0/6	1.2
	14	8.2	1.0	0/7	2/7	5.8
	17	6.0	1.0	0/4	0/4	8.9
		9.1	2.8	17/56	11/39	4.2
t test	P < .05	P < .05	P < .001	P < .001	P < .05	

^aVaccinated with purified pilli of *E. coli* strain 987.

^bMean.

^cTotal.

FIG. 1. Ileum of a pig from a vaccinated gilt. Large mass of E. coli 987 in the lumen, 16 h after challenge. Fluorescent antibody stained frozen section, association index, 1.7. X 27.

FIG. 2. Ileal section of a pig from a control gilt. The E. coli adhere to and uniformly cover the villi, 16 h after challenge. Fluorescent antibody stained frozen section, association index, 5.0. X 27.

FIG. 3. Percentage of pigs with diarrhea born to and suckled by vaccinated gilts compared to those of nonvaccinated gilts during the first 6 days of life.

IMMUNIZATION AGAINST ENTEROTOXIGENIC ESCHERICHIA COLI
INFECTION BY VACCINATION WITH PURIFIED PILI

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Introduction

Bacterial pili are elongated, nonflagella appendages radiating outward from the bacterial cell envelope. The role(s) that the pili of Escherichia coli play in virulence has become the subject of considerable research. Various lines of evidence have indicated that some pili on enterotoxigenic E. coli (ETEC) strains act as colonization factors facilitating adhesion to intestinal mucosa. For example, Smith and Linggood (11) showed that K88, a plasmid mediated pilus (9,13), conferred upon some E. coli strains an ability to colonize the porcine small intestine. Later, Jones and Rutter (5) showed that K88 acted as a colonization factor by permitting bacteria which possess it to adhere to porcine small intestinal mucosa. Two other pili of some porcine ETEC strains appear to function in the small intestines of pigs in a manner analogous to K88. These are the K99 pilus (3,6) which is also genetically determined by a plasmid (12), and the 987 pilus (987P) (4) of unknown genetic origin. Another pilus called type 1 (1) possessed by a human ETEC strain H10407, may also function as a colonization and adhesive factor (2).

It is reasonable to expect that prevention of intestinal colonization will prevent E. coli induced diarrheal disease. Therefore, immunization with purified colonization pili should prevent colonization by inhibiting adhesion and subsequently preventing diarrheal disease. This approach has been successfully applied to experimentally induced diarrheal disease produced by K88+ (10) and 987P+ (Nagy et al. in preparation) ETEC.

The objectives of the work reported here were: (a) to determine whether vaccination with K99 pili protects pigs against infection by a K99+ ETEC, (b) to confirm our initial finding that 987P vaccination protects against infection by strain 987 and to extend this observation to see whether protection is conferred against another 987P+ ETEC which is otherwise serologically unrelated to 987, and (c) to determine if vaccination with heterologous pili will protect against infection (e.g., vaccination with K99 pili protecting against infection by a 987P+ ETEC).

Materials and Methods

E. coli strains and challenge inocula. The three *E. coli* strains used were 431 (0101:K30,93:NM), 987 (09:K103;987P:NM), and 74-5208 (030:K101;987P:NM). Strains 987 and 74-5208, which both undergo phase variations of piliation, were plated onto blood agar plates and piliated clones selected as previously described (8). Challenge inocula were prepared by inoculation of flasks of trypticase soy broth (TSB) with overnight cultures of 431 or piliated 987 grown in tubes of TSB without shaking at 37° C or for 74-5208 directly from a piliated type colony. Flasks were incubated 18-20 hours at 37° C without shaking. Bacterial cells were collected by centrifugation at 7,000 $\times g$ for 10 min and re-suspended in fresh TSB containing 20% sterile glycerol to the following concentrations (987 = 2.5×10^7 /ml, 74-5208 = 5×10^{10} /ml, 431 = 5×10^{10} /ml) and aliquots stored at -70° C.

Vaccines. K99 was prepared as previously described (3) from a laboratory *E. coli* K12 strain harboring the K99 plasmid originally from *E. coli* strain B41. The pili of strain 987 were prepared from a piliated phase variant of strain 987 by a modification of the method of Brinton (in preparation). Both pili were shown to be homogenous by routine physico-chemical methods (3, Brinton et al., in preparation). Purified pili were adjusted to 0.9 mg/ml in saline (0.15 M NaCl) and dialyzed against saline containing 0.05% formaldehyde. Ten ml aliquots of each pilus preparation were stored at 4° C until used for vaccination. Also included in the vaccine battery was a control vaccine consisting of 0.05% formaldehyde in saline. Pregnant swine (gilts) were obtained approximately 3 weeks prior to farrowing. Each gilt was vaccinated twice subcutaneously in the neck with 10 ml of the same vaccine, with the first dose given 16-29 days and the second dose given 5-17 days, prior to farrowing.

Experimental infections and the observations. At birth, pigs were separated from the dam until all were born. They were weighed and returned to the dam for 30 min (for pigs to be challenged with strain 74-5208) or 6-18 hours (for pigs to be challenged with 987 or 431) and allowed to suckle. The pigs were then challenged intragastrically with 20 ml of fresh TSB containing 0.2 ml of the appropriate challenge inoculum. Challenged pigs remained with their dams and were observed daily for 5 days. Data concerning diarrhea and death was recorded. In addition to these data, on the first day (16-29 hours post challenge), the most severely affected pig in each litter (i.e., the pig that lost the most weight or when no weight loss occurred, the pig which gained the least weight) was sacrificed and a 10 cm portion of ileum was removed 10-20 cm from the ileocecal junction to determine the number of viable challenge organisms at this locale. A 1 cm portion of ileum adjacent to

the above portion was also removed, embedded in methylcellulose and used to determine the degree of bacterial adhesion to ileal mucosa by a fluorescent antibody staining technique (7). Adhesion (association index) was based on a 1 to 5 scale depending upon the intensity and location of staining with 1 indicating no adhesion and 5 indicating maximal adhesion.

Results

For the purpose of discussion, the term homologous vaccine will be used to indicate the vaccine which contains the same pilus as the one possessed by the challenge organism and heterologous vaccine will refer to the pilus vaccine which contains a different pilus than the challenge organism. Saline plus formaldehyde vaccine will hereafter be referred to as control.

E. coli 431 (K99+) challenge. Data concerning intestinal colonization, adhesion of bacteria to intestinal mucosa, death, and weight gain for pigs from all three vaccine groups challenged with strain 431 are summarized in Table 1.

None of the 41 pigs in the homologous vaccine (K99) group that were challenged with 431 died. In contrast, 30% of the pigs in the heterologous vaccine group died and 40% of the pigs in the control group died. The prevalence of diarrhea amongst pigs of the three vaccine groups is shown in Figure 1. On day 1, fewer pigs in the homologous vaccine group had diarrhea than those of the other two groups. By day 4, none of these pigs had diarrhea, while approximately 45% of the surviving pigs in the control group and approximately 80% of the surviving pigs in the heterologous vaccine group still had diarrhea. In the heterologous vaccine group it took until day 4 till a decrease in diarrhea occurred.

The prolonged diarrhea in the heterologous vaccine and control groups is also reflected in the weight gain of surviving pigs (Table 1). There were statistically fewer ($P < .01$) viable 431 bacteria in the ilea of pigs in the homologous vaccine group than in the heterologous vaccine or control groups. However, all pigs sampled in all three vaccine groups were by our operational definition considered to be colonized (i.e., $> 10^5$ challenge organisms per 10 cm of intestine). The degree of adhesion of the challenge strain to ileal mucosa was determined by using fluorescent-antibodies to stain ileal sections. Sections from pigs from all three vaccine groups were indistinguishable from each other with the mean association indices between 4 and 5 indicating good adhesion in all cases. Therefore, even though the homologous vaccine, K99, protected against death and diarrhea after challenge with strain 431, it did not

Table 1. Response of pigs from the three vaccine groups to challenge with E. coli strain 431

Vaccine	1 day after challenge		5 days after challenge	
	Mean E. coli 431/10 cm ileum	Mean association index	Death/total	Weight gain of survivors (%)
K99 (n = 5) ^a	2.4×10^9	$(1.6 \times 10^9 - 3.36 \times 10^9)^b$	0/41	37
987 (n = 6)	5.37×10^9	$(1.7 \times 10^9 - 1.0 \times 10^{10})$	9/30	-3.6
Control (n = 5)	6.7×10^9	$(4.2 \times 10^9 - 1 \times 10^{10})$	14/35	9.1

^a n = number of litters.

^b Range in parenthesis.

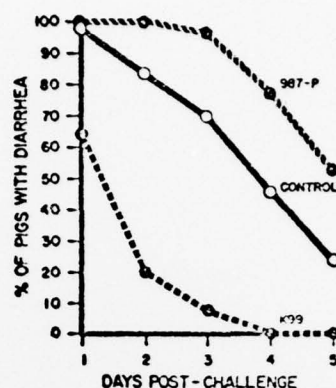


Figure 1. Prevalence of diarrhea among pigs challenged with *E. coli* strain 431 as described in Materials and Methods. Each point represents the percent of surviving pigs with diarrhea in an entire vaccine group on the day of measurement.

prevent colonization and adhesion to the terminal ileum by this strain one day after challenge. Since this was an unexpected result, the following experiments were performed. Two K99 vaccine litters and one 987P vaccine litter were challenged with 431 as usual. On a daily basis for 5 days, one pig per litter was sacrificed (the pig selected was the most severely affected based on weight change). Five 10 cm portions of small intestines were removed at equal intervals starting at the usual ileal location and moving cranially until 20 cm posterior to the ligament of Treitz. These segments were evaluated for viable challenge bacteria and adhesion; both indicators of colonization. Pigs in the homologous vaccine group were colonized for fewer days than pigs in the heterologous vaccine group (~ 2 days compared to ~ 5 days) and colonization was less extensive (only the posterior 2 segments of pigs in the homologous vaccine group were colonized on day 1, while the posterior 3 segments in the heterologous vaccine group were colonized on day 1).

E. coli 987 and 74-5203 challenges. Previously, we demonstrated that vaccination of pregnant gilts with purified 987 pili protected pigs against experimentally induced diarrheal disease caused by strain 987 (Nagy et al. in preparation). This experiment has been repeated and extended to see whether vaccination with 987P protects against disease produced by a serologically unrelated strain 74-5203 (except for pili) possessing the 987 pilus. Evaluation of these experiments will be limited to data concerning death and diarrhea.

1. E. coli 987 challenge. None of the 28 pigs in the homologous vaccine group (987P) challenged with strain 987 died, while 17% of the pigs in the heterologous vaccine group and 27% of the pigs in the control group died. Figure 2 shows the prevalence of diarrhea among pigs challenged with 987. On day 1, approximately 18% of the pigs in the homologous vaccine group had diarrhea, compared to 84 and 90% of the pigs in the other two groups. By day 3, none of the pigs in the homologous vaccine group had diarrhea, while 50 and 65% of the surviving pigs in the other two groups still had diarrhea.

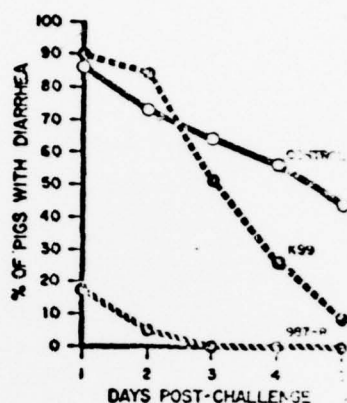


Figure 2. Prevalence of diarrhea among pigs challenged with E. coli strain 987. Conditions are the same as in Figure 1.

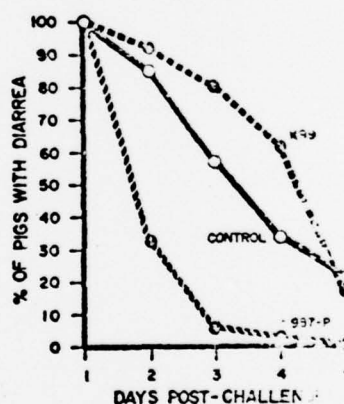


Figure 3. Prevalence of diarrhea among pigs challenged with E. coli strain 74-5208. Conditions are the same as in Figure 1.

11. E. coli 74-5208 challenge. Three percent of (1 of 37) pigs in the homologous vaccine group (987P) died after challenge with strain 74-5208. However, 18% of the pigs in the heterologous vaccine group and 15% of the pigs in the control group died. The prevalence of diarrhea among pigs (Figure 3) are similar to that observed with 987 challenge (Figure 2) except that on day 1 all pigs in the three vaccine groups had diarrhea.

Discussion

The data presented here demonstrate that vaccination of pregnant gilts with purified pili provides passive protection of their suckling pigs against diarrheal disease caused by an ETEC strain possessing the homologous pilus. Pigs born to gilts that had been vaccinated with K99 had a lower incidence of death, decreased incidence and duration of diarrhea, and better weight gain than pigs born to 987P or control vaccinated gilts when challenged with the K99 strain 431. Similarly, pigs born to 987P vaccinated gilts when challenged with either strains 987 or 74-4208 had lower incidences of death and equal or lower incidences and durations of diarrhea than their K99 vaccinated counterparts. In no instance did a pilus confer protection upon pigs challenged with a totally heterologous strain. Thus, for example, vaccination with K99 did not protect against diarrheal disease caused by strain 987.

The mechanism whereby protection occurs cannot be readily ascertained from the data presented. However, since the vaccinating agents used were purified pili, it is assumed that immunity arises in pigs from the acquisition of anti-pilus immunoglobulins via ingested colostrum. The correlation of protection with high antibody titers against homologous pili (and not with those against heterologous pili, O, and polysaccharide K antigens) in serum and colostrum of vaccinated dams (unpublished data) is consistent with this assumption. Once in the small intestine, these antibodies could act in a variety of ways. The mechanism that we favor and which is most consistent with the hypothesized action of pili is that the antibodies prevent bacterial colonization by inhibiting and/or reversing bacterial adhesion. It is possible that the antibodies may also act by agglutinating or opsonizing the challenge bacteria. These mechanisms should not be considered as mutually exclusive. The possibility that the purified pilus preparations may have contained some undetected immunizing antigen(s) which accounts for the protection is recognized. However, this is unlikely because vaccination with either pilus preparation protected against challenge with strains which (except for pili) were antigenically unrelated to the strains used for vaccine production.

The data presented compliments the studies of Rutter and Jones (10) who showed that gilts vaccinated with K89 also protect their

suckling pigs against diarrhea caused by K88+ ETEC, and the studies of Brinton (in preparation) who showed that human volunteers vaccinated with Neisseria gonorrhoeae pili were protected against intraurethral infection with the homologous strain of gonococci. We suggest that many other bacterial infections of mucosal tissues may be controllable with the use of pilus vaccines.

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