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## Vaccination of Pregnant Dams with Intimin<sub>O157</sub> Protects Suckling Piglets from *Escherichia coli* O157:H7 Infection

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**Cattle are important reservoirs of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 that cause disease in humans. Both dairy and beef cattle are asymptotically and sporadically infected with EHEC. Our long-term goal is to develop an effective vaccine to prevent cattle from becoming infected and transmitting EHEC O157:H7 to humans. We used passive immunization of neonatal piglets (as a surrogate model) to determine if antibodies against EHEC O157 adhesin (intimin<sub>O157</sub>) inhibit EHEC colonization. Pregnant swine (dams) with serum anti-intimin titers of  $\leq 100$  were vaccinated twice with purified intimin<sub>O157</sub> or sham-vaccinated with sterile buffer. Intimin<sub>O157</sub>-specific antibody titers in colostrum and serum of dams were increased after parenteral vaccination with intimin<sub>O157</sub>. Neonatal piglets were allowed to suckle vaccinated or sham-vaccinated dams for up to 8 h before they were inoculated with  $10^6$  CFU of a Shiga toxin-negative (for humane reasons) strain of EHEC O157:H7. Piglets were necropsied at 2 to 10 days after inoculation, and intestinal samples were collected for determination of bacteriological counts and histopathological analysis. Piglets that ingested colostrum containing intimin<sub>O157</sub>-specific antibodies from vaccinated dams, but not those nursing sham-vaccinated dams, were protected from EHEC O157:H7 colonization and intestinal damage. These results establish intimin<sub>O157</sub> as a viable candidate for an EHEC O157:H7 antitransmission vaccine.**

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a common cause of bloody diarrhea and the most common cause of hemolytic-uremic syndrome in humans in the United States (1). EHEC strains can be foodborne pathogens, and cattle are important reservoirs of EHEC O157:H7 strains. All EHEC strains produce cytotoxins called Shiga toxins (Stx1 and Stx2), previously called Shiga-like toxins (SLT-1 and SLT-2) or verotoxins (VT1 and VT2). Many EHEC strains, including EHEC O157:H7, can attach intimately to host cell membranes and efface microvilli and cytoplasm in a pattern referred to as an attaching-and-effacing (A/E) lesion (18). EHEC intimin is an outer membrane protein adhesin encoded by the *eae* gene of EHEC (16) that is required for intestinal colonization and for A/E activity of EHEC O157:H7 in piglets (6, 8, 9, 19) and neonatal calves (6). Antibodies against intimin<sub>O157</sub> significantly reduce the adherence of *E. coli* O157:H7 strains, as well as certain related strains, to HEp-2 cells (11). Based on this observation, we hypothesize that a vaccine that contains intimin<sub>O157</sub> may induce an immune response in cattle that will prevent or decrease the level of intestinal colonization by *E. coli* O157:H7. We speculate that the resultant diminution in fecal shedding of EHEC O157:H7 by these animals could, in turn, lead to a decrease in the transmission of EHEC O157:H7 and other A/E *E. coli* strains to humans.

In the study reported here, we tested the concept that anti-intimin antibodies can protect animals from colonization with

EHEC O157:H7. For that purpose, we selected neonatal piglets as the bacterial challenge model. Although pigs have not been identified as a common reservoir of EHEC O157:H7 strains, the neonatal piglet EHEC infection model has been used to demonstrate the critical role of intimin in EHEC O157:H7 infections (6, 19). Furthermore, neonatal suckling piglets are a good surrogate model for determining if antibodies against intimin prevent EHEC colonization and disease.

The objectives of this investigation were twofold. First, we sought to determine whether parenteral vaccination of pregnant swine (first-litter dams) with intimin<sub>O157</sub> elicits anti-intimin<sub>O157</sub> antibodies in their serum and colostrum. Second, we wanted to assess whether piglets that ingest colostrum (first milk) containing anti-intimin<sub>O157</sub> antibodies are passively immunized and protected from EHEC O157:H7 infection and disease. To prevent the development of Stx-mediated systemic disease in the neonatal animals, we challenged them with an Stx-negative *E. coli* O157:H7 strain. We felt confident that substitution of this Stx<sup>-</sup> strain for wild-type EHEC O157:H7 (which was done for humane purposes) would not compromise our results because both Stx<sup>+</sup> and Stx<sup>-</sup> *E. coli* O157:H7 strains similarly colonize and cause A/E lesions in the large intestines of suckling piglets (3).

### MATERIALS AND METHODS

**Intimin<sub>O157</sub> purification.** The histidine-tagged intimin<sub>O157</sub> protein RIHisEae was purified as described previously (11, 20) and used for vaccination of pregnant dams and for anti-intimin<sub>O157</sub> dot blot assays. RIHisEae is a histidine-tagged version of the entire intimin protein from EHEC O157:H7 strain 86-24 minus the N-terminal 35 amino acids, which are thought to be part of a cleaved N-terminal signal sequence (13, 20). For Western blot analysis of colostrum samples, a histidine-tagged N-terminal two-thirds fragment of intimin and a histidine-

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tagged C-terminal one-third fragment of intimin were purified as described previously (11).

**Animals and vaccinations.** Three crossbred pregnant dams (first-litter dams; dams A, C, and E) were vaccinated intramuscularly with intimin<sub>O157</sub> mixed with TiterMax Gold adjuvant (500 µg of intimin/dose) at 2 and 4 weeks prior to farrowing (giving birth). Three pregnant dams (dams B, D, and F) were sham vaccinated with Tris-buffered saline (TBS) mixed with adjuvant. Piglets naturally farrowed at the National Animal Disease Center by these dams were allowed to suckle colostrum before inoculation with *E. coli* O157:H7. One intimin<sub>O157</sub>-vaccinated dam (dam C) had complications at farrowing and may not have allowed all of the piglets to nurse freely prior to inoculation.

**Colostrum and blood samples.** Colostrum was collected from each dam at parturition. Blood samples were collected from the dams prior to the first vaccination (prevaccination) and at 7 to 11 days after farrowing when dams were euthanized (postvaccination). Blood samples were collected from all of the piglets in the last two litters tested, one vaccinated litter (dam E) and one sham-vaccinated litter (dam F), immediately before they were inoculated with *E. coli* O157:H7. Colostral whey and sera were prepared as previously described and stored at -80°C until tested (4).

**Inoculation with *E. coli* O157:H7.** All piglets were inoculated with 10<sup>6</sup> CFU of Stx-negative *E. coli* O157:H7 strain 87-23 (17, 25) administered via stomach tube after all of the piglets had nursed and before the piglets were 8 h old. Inocula were prepared and stored as previously described (5). Piglets were returned to the dam immediately after inoculation and observed clinically every 4 to 8 h.

**Necropsy.** Piglets were euthanized with sodium pentobarbital and necropsied at 2 to 10 days postinoculation (p.i.). Piglets were randomly assigned to each of the necropsy groups. At necropsy, sections of the cecum were collected aseptically for determination of bacterial counts and sections of the distal colon, spiral colon, cecum, and ileum were collected for histopathologic examination and immunoperoxidase (horseradish peroxidase [HRPO]) staining with anti-O157:H7 antibody (see below).

**Bacteriologic examination.** Sorbitol-negative *E. coli* O157:H7 bacteria were quantitated on sorbitol-MacConkey agar. Selected sorbitol-negative colonies were tested for O157:H7 antigens by latex agglutination assay (5). Bacterial colonization was defined as ≥10<sup>6</sup> CFU of inoculum bacteria/g of cecal tissue. Because dilutions made in counting did not permit recovery of <10<sup>4</sup> CFU of *E. coli* per g, tissues from which the inoculum strain was not recovered were recorded as having <10<sup>4</sup> CFU/g of tissue.

**Histologic studies.** Tissues were fixed in neutral buffered 10% formalin, processed by routine methods, sectioned, and stained with hematoxylin and eosin (H&E). Stained tissue slides were coded and examined by light microscopy. A/E lesions were scored from 0 (negative) to 4+ (≥50% of villi or surface epithelium affected) as previously described (5).

**Immunoperoxidase staining with anti-O157:H7.** O157:H7 bacteria were identified in paraffin-embedded, formalin-fixed tissues by indirect immunoperoxidase staining with anti-O157:H7 as previously described (5).

**Anti-intimin<sub>O157</sub> dot blot assay.** Anti-intimin<sub>O157</sub> titers in swine serum and colostrum were determined with a dot blot enzyme-linked immunosorbent assay. Purified intimin<sub>O157</sub> (100 µl/dot; 12 µg of intimin/ml in 0.1 M Tris-154 mM NaCl, pH 7.4 [TBS]) was applied to nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.) with a 96-well Millipore apparatus (Millipore Corp., Bedford, Mass.). Unbound intimin was removed and additional binding sites on the filters were blocked by three washes with 0.05% Tween 20 (Sigma, St. Louis, Mo.) in TBS (TBS-T). All remaining incubations were for 1 h at 22°C. Diluted serum or colostrum (100 µl of serial 10-fold dilutions ranging from 10<sup>-2</sup> to 10<sup>-4</sup> or 10<sup>-5</sup> per well) in TBS-T was added, and the mixture was incubated. After three washes with TBS-T, filters were removed from the Millipore apparatus and washed for an additional 10 min in TBS-T. Total bound immunoglobulin was detected by sequential incubations with peroxidase-labeled goat anti-swine immunoglobulin G (heavy and light chains; Kirkegaard & Perry Laboratories, Gaithersburg, Md.) and substrate (0.5 mg of 4-chloro-1-naphthol per ml, 0.015% H<sub>2</sub>O<sub>2</sub>, and 16.7% methanol in TBS). The anti-intimin<sub>O157</sub> titer is the reciprocal of the highest dilution of serum or colostrum that yielded a purple (peroxidase-positive) precipitate on a filter.

**Adherence assays.** The ability of porcine colostrum from intimin-vaccinated dam A and sham-vaccinated dam B to inhibit the adherence of *E. coli* strain 87-23 to HEp-2 (human laryngeal epithelial) cells was assessed as described previously (11). Briefly, colostrum samples were diluted 1:27 into 0.3 ml of adherence medium (Eagle minimum essential medium, 1% mannose, 0.4% sodium bicarbonate), mixed with 4 × 10<sup>6</sup> bacteria of *E. coli* strain 87-23, and incubated for 35 min at 37°C. The bacterium-colostrum mixtures were added to monolayers of HEp-2 cells that were incubated for 3 h at 37°C and washed twice. Fresh adherence medium with colostrum was added for a second 3-h incubation,

TABLE 1. Anti-intimin<sub>O157</sub> titers in serum and colostrum of pregnant dams after vaccination with intimin<sub>O157</sub>

Vaccination and dam	Anti-intimin <sub>O157</sub> titer		
	Serum		Colostrum
	Prevaccination	Postvaccination	
Intimin <sub>O157</sub>			
A	≤100	≥10,000	≥100,000
C	≤100	≥10,000	≥100,000
E	≤100	≥10,000	≥100,000
Sham (TBS)			
B	≤100	≤100	≤100
D	≤100	≤100	≤100
F	≤100	≤100	≤100

after which the cells were washed and stained as described previously. Adherence was quantitated by determining the number of bacteria per HEp-2 cell, with at least 250 HEp-2 cells and at least 600 bacteria counted per sample. The adherence inhibition assays of these colostrum samples were done three times on different days.

**Western blot analysis.** Colostrum samples from dams A and B were subjected to Western blot analysis for reactivity with whole-cell lysates of EHEC and for reactivity with purified intimin protein. Whole-cell bacterial lysates of wild-type EHEC O157:H7 strain 86-24 (14) and its isogenic *eae*<sup>-</sup> mutant derivative 86-24 *eae*Δ10 (19) were prepared and loaded onto sodium dodecyl sulfate-polyacrylamide gels as described previously (11). Proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were blotted onto nitrocellulose and processed for immunoblot analysis with visualization by chemiluminescence as described previously (11). A 1:7,500 dilution of each colostrum sample was used, followed by an anti-swine secondary antibody (Sigma). Western blots were scanned, and images of the blots were arranged and labeled with Adobe Photoshop version 5.0.2 for Macintosh.

## RESULTS

**Anti-intimin<sub>O157</sub> titers.** As shown in Table 1, the serum anti-intimin<sub>O157</sub> titers of all of the pregnant dams were ≤100 before they were vaccinated. At the time of farrowing, all of the intimin<sub>O157</sub>-vaccinated dams had colostral anti-intimin<sub>O157</sub> titers of ≥100,000 and all of the sham-vaccinated dams had titers of ≤100. The serum anti-intimin<sub>O157</sub> titers of the vaccinated dams after they had farrowed were >10,000, but those of the sham-vaccinated dams were ≤100. All seven of the piglets that nursed a vaccinated dam (dam E) and were at least 2 h old when they were inoculated with *E. coli* O157:H7 had serum anti-intimin<sub>O157</sub> titers of ≥10,000 at the time they were challenged. Two piglets in this same litter that were inoculated before they were 2 h old had a titer of 100. All eight of the piglets nursing sham-vaccinated dam F had titers of <100.

**Adherence of *E. coli* strain 87-23 to HEp-2 cells in vitro in the presence of colostrum.** A small but reproducible difference in the number of adherent bacteria was observed in the presence of colostrum from the intimin<sub>O157</sub>-immunized dam compared to colostrum from the sham-vaccinated dam (35% fewer adherent bacteria in the presence of colostrum from the intimin<sub>O157</sub>-immunized dam, as averaged from the three replicate assays; *P* < 0.05; data not shown). Neither colostrum sample agglutinated the bacteria, as assessed by slide agglutination assays. Further, the growth of strain 87-23 in the presence of colostrum from the intimin<sub>O157</sub>-immunized dam was comparable to that in the presence of colostrum from the

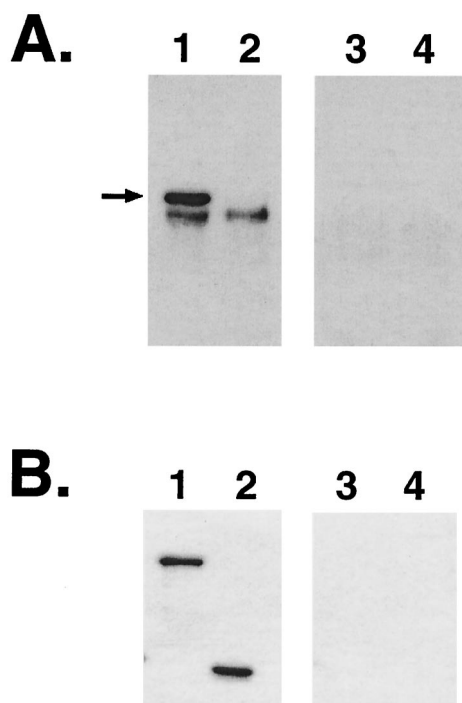


FIG. 1. Western blot analysis of colostrum. Colostrum samples from an intimin-vaccinated dam (lanes 1 and 2) and a sham (TBS)-vaccinated dam (lanes 3 and 4) were assayed by Western blotting. (A) Reactivity of colostrum samples against whole-cell lysates of EHEC O157:H7 strain 86-24 (lanes 1 and 3) and intimin mutant strain 86-24 *eae*Δ10 (lanes 2 and 4). The region of the blot containing intimin (arrow) is shown; the colostrum also reacted with lower-molecular-weight bands in the lysates. The band migrating just below intimin may be a cross-reacting band. (B) Reactivity of colostrum samples with a purified N-terminal two-thirds fragment of intimin (lanes 1 and 3) and a purified C-terminal one-third fragment of intimin (lanes 2 and 4) from EHEC strain 86-24.

sham-vaccinated dam, as determined from colony counts after growth in adherence medium.

**Western blot analysis.** Colostrum samples from one intimin-vaccinated and one sham-vaccinated dam (dams A and B) were also analyzed by Western blotting. The colostrum from the intimin<sub>O157</sub>-vaccinated dam, but not that from the sham-vaccinated dam, reacted with intimin in a whole-cell lysate of EHEC O157:H7 (Fig. 1A). The immune colostrum contained antibodies that recognized N- and C-terminal portions of intimin<sub>O157</sub>, as demonstrated by reactivity against purified intimin fragments (Fig. 1B).

**Clinical and gross pathological observations.** All but one of the piglets remained clinically healthy following inoculation with Stx-negative *E. coli* O157:H7 strain 87-23. One weak and depressed piglet in a vaccinated litter (litter A) had mild diarrhea between 8 and 24 h after inoculation but not between 24 and 48 h when it was necropsied. This pig and two other piglets (one of which was lame) from the same litter had moderate colonic edema and hyperemia when necropsied at 2 or 3 days after inoculation (but none of these three pigs were colonized with the inoculum strain or had A/E lesions).

**Bacterial colonization.** Table 2 shows the numbers of inoculum bacteria that were recovered from the suckling piglets. Most (25 of 30) of the piglets nursing sham-vaccinated dams

were colonized by *E. coli* O157:H7 (i.e., had  $\geq 10^6$  CFU of inoculum bacteria/g of cecal tissue) at 2 to 10 days after inoculation. The five piglets that were not colonized were necropsied at either 2 or 3 days after inoculation. In contrast, only 6 of the 25 piglets nursing intimin<sub>O157</sub>-vaccinated dams were colonized or had A/E lesions at 2 to 10 days after inoculation. Five of these six colonized piglets were from litter C, the litter that might not have had free access to colostrum before challenge (see Materials and Methods). No *E. coli* O157:H7 bacteria (i.e.,  $< 10^4$  CFU of inoculum bacteria/g of cecal tissue) were recovered from 15 (including the 2 piglets in litter E that had low serum anti-intimin<sub>O157</sub> titers) of the 19 piglets suckling vaccinated dams that were not colonized.

**A/E lesions.** Most (25 of 30) piglets nursing the three sham-vaccinated control dams had A/E lesions in the large intestines at 2 to 10 days after inoculation with *E. coli* O157:H7 (Table 2 and Fig. 2). All sham-vaccinated piglets that had  $\geq 10^6$  CFU of O157:H7/g of cecal tissue had A/E lesions. In contrast, only 8 of the 25 piglets nursing intimin<sub>O157</sub>-vaccinated dams had A/E lesions. Three of these piglets had rare O157:H7-positive A/E lesions that were detected only by HRPO staining. No A/E lesions were seen in the two piglets in litter E that had low serum anti-intimin<sub>O157</sub> titers at the time of inoculation. A/E lesions were found in all cecal tissues that had at least  $10^6$  CFU of inoculum bacteria/g of cecal tissue. O157:H7-positive A/E lesions were identified by HRPO in all tissue sections in which A/E lesions were detected by H&E staining. As shown in Fig. 2, the A/E scores of vaccinated piglets with A/E lesions were considerably lower than those of the sham-vaccinated piglets. Five of the piglets that nursed vaccinated dam C, but none of the piglets that nursed the other two vaccinated dams, had A/E lesions that were detected by H&E staining. Two additional piglets from litter C and one piglet from litter A (clinically normal and without gross lesions) had rare O157:H7-positive A/E lesions that were detected only by HRPO staining.

TABLE 2. Effect of vaccination of pregnant dams with intimin<sub>O157</sub> on experimental *E. coli* O157:H7 infection in suckling piglets

Vaccination and litter(s)	No. of piglets positive/no. tested					
	Colonized <sup>a</sup>			A/E lesions in large intestine <sup>b</sup>		
	Total	2-4 days pi	6-10 days pi	Total	3-4 days pi	6-10 days pi
<b>Intimin<sub>O157</sub></b>						
A + C + E	6/25 <sup>c</sup>	5/20	1/5	8/25 <sup>c,d</sup>	6/20 <sup>d</sup>	2/5
A	1/8	1/8	ND <sup>e</sup>	1/8 <sup>d</sup>	1/8 <sup>d</sup>	ND
C	5/8	4/6	1/2	7/8 <sup>d</sup>	5/6 <sup>d</sup>	2/2
E	0/9	0/6	0/3	0/9	0/6	0/3
<b>Sham (TBS)</b>						
B + D + F	25/30	17/22	8/8	26/30	18/22	8/8
B	8/11	6/9	2/2	9/11	7/9	2/2
D	9/9	6/6	3/3	9/9	6/6	3/3
F	8/10	5/7	3/3	8/10	5/7	3/3

<sup>a</sup>  $\geq 10^6$  CFU of inoculum bacteria/g of cecum.

<sup>b</sup> A/E lesions identified as O157:H7<sup>+</sup> by immunostaining.

<sup>c</sup> Significantly different from sham-vaccinated piglets ( $P < 0.05$  [chi-square analysis]).

<sup>d</sup> A/E lesions were detected only by HRPO, and not by H&E staining, in one piglet in litter A and two in litter C.

<sup>e</sup> ND, not done.

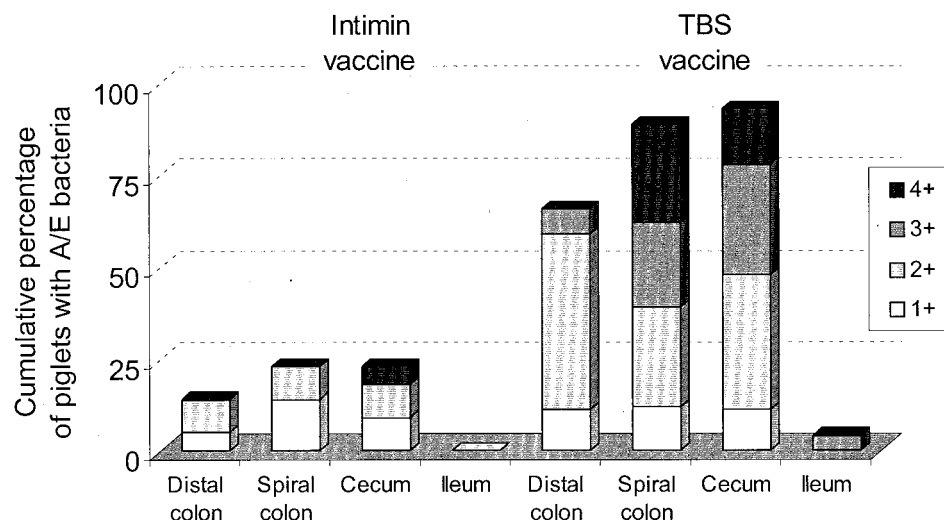


FIG. 2. Effect of intimin<sub>O157</sub> vaccination of pregnant gilts on the distribution and intensity of A/E lesions in suckling piglets at 3 to 10 days p.i. A/E scores reflect the extent of villi or surface epithelium affected, as estimated by examination of H&E-stained sections. Scoring: 1+, detected only in HRPO-stained sections; 2+, <10% of the villi or surface epithelium affected; 3+, 10 to 50% of the villi or surface epithelium affected; 4+,  $\geq$ 50% of the villi or surface epithelium affected.

## DISCUSSION

A vaccine preparation consisting of intimin<sub>O157</sub> mixed with TiterMax Gold adjuvant and administered parenterally at 2 and 4 weeks prior to farrowing was immunogenic in pregnant swine. Most suckling piglets that ingested colostrum containing anti-intimin<sub>O157</sub> antibodies from intimin<sub>O157</sub>-vaccinated dams were not colonized and did not develop intestinal lesions when they were challenged with *E. coli* O157:H7. The two piglets from litter E that were inoculated before they were 2 h old may have had a serum anti-intimin<sub>O157</sub> titer of only 100 at the time of inoculation because they had not yet had time to absorb antibody from colostrum into the serum. They might have had higher serum titers if sampled later. This is supported by the observation that they were protected from colonization after challenge. The protective antibodies were most likely transmitted via colostrum because immunoglobulins are not transmitted in utero to the fetuses of healthy sows.

Colostrum from the one intimin<sub>O157</sub>-vaccinated dam that we tested, but not that from a sham-vaccinated dam, inhibited the adherence of *E. coli* O157:H7 strain 87-23 to HEp-2 cells. This colostrum contained antibodies against the C-terminal third of intimin, the region that is known to be critical for interaction with mammalian cells (7, 10). The inhibitory effect on adherence did not appear to be due to agglutination or inhibition of bacterial growth by the colostrum. These observations support the hypothesis that the presence of antibodies against intimin<sub>O157</sub> in the intestines of suckling piglets inhibits bacterial adherence. The effects of antibodies on adherence in vitro (35% inhibition) were weaker than those observed in vivo (seven of eight piglets were protected from adherence and colonization).

None of the pregnant dams in this study had preexisting anti-intimin<sub>O157</sub> titers of >100, but all of the intimin<sub>O157</sub>-vaccinated dams had colostral anti-intimin<sub>O157</sub> titers of  $\geq$ 100,000 at farrowing. However, one pregnant dam was excluded from an earlier pilot study because it had an anti-intimin<sub>O157</sub> titer of

1:10,000 prior to sham vaccination (with TBS and adjuvant) and a colostral anti-intimin<sub>O157</sub> titer of 100,000 at farrowing. The presence of anti-intimin<sub>O157</sub> antibodies in a nonvaccinated pig is consistent with previous reports that some porcine *E. coli* isolates have A/E activity and/or contain the *eae* gene, which encodes intimin (15, 21, 22, 26), and could be reservoirs of EHEC associated with human infections (2, 23, 24).

In this study, as in previous studies, the presence of A/E lesions containing O157: H7-positive bacteria correlated with the number of inoculum bacteria recovered from the ceca of piglets inoculated with *E. coli* O157:H7 bacteria. A/E lesions were seen in most piglets that had  $\geq$ 10<sup>6</sup> CFU of O157:H7/g of cecum but rarely in ones with fewer bacteria.

Consistent with our earlier observations (3), most of the sham-vaccinated suckling piglets were colonized and had intestinal lesions by 3 days p.i. but showed no signs of disease. It was somewhat surprising that all of the sham-vaccinated suckling piglets examined between 6 and 10 days p.i. were colonized with O157:H7 bacteria and had A/E lesions. Apparently, *E. coli* O157:H7 was not cleared from the large intestines of neonatal piglets (based on high cecal counts) as rapidly as it is thought to be cleared from some EHEC-infected humans (as estimated by negative fecal cultures). The inability to recover EHEC O157:H7 from the feces of some human patients with bloody diarrhea or hemolytic-uremic syndrome has been attributed to clearance of the causative EHEC bacteria by the time the patients seek medical care and samples are collected for culture. It was encouraging to see that piglets nursing the vaccinated dams were still not colonized at 9 to 10 days after inoculation.

The long-term goal of our research is to develop a means for reducing the transmission of *E. coli* O157:H7 from cattle to humans. The concept that antibodies to intimin<sub>O157</sub> may block colonization of animals with O157:H7 was strongly supported by the passive immunization studies with suckling piglets described here. Indeed, the results of these experiments clearly

demonstrated that piglets that ingested maternal antibodies against intimin<sub>O157</sub> were protected from colonization and intestinal damage following experimental inoculation with an intimin-producing, nontoxicogenic *E. coli* O157:H7 strain. These results establish intimin<sub>O157</sub> as a viable candidate for an EHEC O157:H7 antitransmission vaccine. Recent evidence showing that active immunization of mice with intimin prevents colonization by the intimin-positive mouse pathogen *Citrobacter rodentium* (12) further bolsters the idea of using intimin in vaccines against EHEC pathogens. For humane reasons, we have not tested the vaccine against Stx-positive strains in neonatal piglets because they are highly sensitive to Stx-mediated neurological disease (3). However, since cattle appear to be resistant to Stx-mediated disease, we will use Stx-positive EHEC strains in future studies to determine if anti-intimin<sub>O157</sub> antibodies can also prevent EHEC colonization and transmission in cattle.

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