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Experimental Infection of Newly Weaned Pigs with Human and Porcine Strains of *Serpulina pilosicoli*

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Cultures of *Serpulina pilosicoli* 95/1000, isolated from a pig with porcine intestinal spirochetosis (PIS), and *S. pilosicoli* WesB, isolated from an Aboriginal child with diarrhea, were used to infect 5-week-old newly weaned pigs. Four of 12 pigs infected with strain 95/1000 and 2 of 12 pigs infected with strain WesB became colonized and developed watery, mucoid diarrhea within 2 to 11 days postinfection. Affected pigs all had moderate subacute mucosal colitis, with gross and histological changes similar to those previously reported in both natural and experimentally induced cases of PIS. Silver-stained histological sections of the colon and cecum from affected pigs demonstrated spirochetes within dilated intestinal crypts, where they were associated with neutrophilic exocytosis and mucus secretion. Sections from one pig infected with strain 95/1000 showed large numbers of spirochetes attached by one end to the colonic epithelium, a feature consistent with PIS. This study confirms the role of *S. pilosicoli* in the etiology of PIS and provides evidence that *S. pilosicoli* strains of human origin have pathogenic potential in an animal model.

Porcine intestinal spirochetosis (PIS) is a diarrheal disease which occurs in pigs between 4 and 20 weeks of age but is most commonly encountered in the immediate postweaning period (9). The major clinical signs associated with PIS include weight loss, poor growth rate, and diarrhea with occasional flecks of blood (25). The attachment of large numbers of intestinal spirochetes by one cell end to the colonic epithelium is a pathognomonic histological feature of the condition (12). Strain P43/6/78 was used in the first recorded experimental reproduction of intestinal spirochetosis in pigs (25); this strain recently was identified as *Serpulina pilosicoli*, a new species of intestinal spirochete (27).

Human intestinal spirochetosis (HIS), a condition also characterized by the attachment of spirochetes by one end to the colonic epithelium, has been recognized for many years (10, 15). Some reports have questioned whether HIS is specifically associated with gastrointestinal illness (21), while others have associated it with a variety of intestinal disorders but particularly with rectal bleeding and chronic diarrhea (3, 4, 7, 13, 16). When isolates from some of the latter studies have been examined in detail, they have been identified as *S. pilosicoli* (17). In contrast, it appears that in cases in which spirochetes have been recorded in healthy individuals, they have been identified as *Brachyspira aalborgi*, a distinct species considered to be a nonpathogenic commensal (11, 21). HIS caused by *S. pilosicoli* occurs most commonly in individuals in developing countries, in Australian Aborigines, in homosexual males, and in AIDS patients (17).

Since the initial descriptions of Taylor and coworkers (25) and Andrews and Hoffman (1), there have been no other reports of the successful reproduction of diarrhea and the end-on attachment of spirochetes in conventional pigs infected with strains now recognized as *S. pilosicoli*. In 1-day-old specific-pathogen-free (SPF) chicks orally challenged with either human or porcine strains of *S. pilosicoli*, we previously have induced diarrhea and retarded growth rates and have caused the attachment of large numbers of spirochetes by one end to the cecal epithelium (26). This confirmed that *S. pilosicoli* strains can cross species boundaries and cause disease. In the present study we report on the infection and induction of disease in newly weaned conventional pigs with both a human and a porcine strain of *S. pilosicoli.*

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MATERIALS AND METHODS

Source of animals. Thirty-two newly weaned 4-week-old healthy pigs were obtained from a local piggery known to be free from swine dysentery and PIS. To confirm that the pigs were free from intestinal spirochetes, fecal samples from each pig were cultured. Samples from each pig were plated onto Trypticase soy agar supplemented with 5% defibrinated ovine blood, spectinomycin (400 μg/ml), vancomycin (25 μg/ml) and colistin (25 μg/ml) and incubated at 37°C for 5 days in an atmosphere of 94% H_2 and 6% CO_2.

Diets and experimental design. The pigs were weighed and randomly divided into three groups (A, B, and C) which were housed separately. Each group was further subdivided into two subgroups which were maintained ad libitum on one of two weaner diets (mean weights and standard deviations: A1 to A6, 8.0 ± 0.7; A7 to A12, 7.9 ± 0.6; B1 to B6, 8.4 ± 0.7; B7 to B12, 7.7 ± 1.5; C1 to C4, 7.5 ± 0.2; C5 to C8, 7.8 ± 1.3). Pigs A1 to A6, B1 to B6, and C1 to C4 were fed a pelleted Western Australian antibiotic-free commercial pig weaner ration (diet 1). The dry-weight composition of the major ingredients was 62% whole wheat, 15% dehulled lupin, 11.5% animal protein, and 3% peas. The remaining pigs were fed an unpelleted diet (diet 2) based on cooked rice and lupins (dry-weight composition of major ingredients: 64% cooked rice, 15% dehulled lupin, and 13% animal protein). Both diets had a digestible energy content of 14.7 MJ/kg, contained 20% crude protein on a dry-matter basis, were highly fermentable, and have been shown to predispose pigs to development of swine dysentery when orally challenged with *Serpulina hyodysenteriae* (23).

The spirochetes used to infect the pigs were propagated in anaerobic Trypticase soy broth medium (14), as previously described (26). Pigs in group A (n = 12) were inoculated with porcine *S. pilosicoli* 95/1000, originally isolated from a 16-week-old pig in Western Australia which had died with postmortem lesions consistent with PIS. Pigs in group B (n = 12) were inoculated with human *S. pilosicoli* WesB, isolated from an Australian Aboriginal child with diarrhea (16, 26). Both strains previously were confirmed to be *S. pilosicoli* by their grouping in multilocus enzyme electrophoresis (MEE) (17) and by their positive reactions in a PCR specific for this group of organisms (22). Pigs in group C (n = 8) were dosed with 100 ml of sterile Trypticase soy broth medium and acted as uninoculated controls. Each pig in the two test groups was dosed by stomach tube with approximately 10^{10} spirochetes daily for three consecutive days. The pigs were monitored daily for signs and symptoms of disease, and fecal swabs were obtained in accordance with the schedule shown in Table 1. The final weight of each of the pigs was obtained 11 days postinfection (p.i.), and the animals then were euthanized by intravenous phenobarbitone injection.

Preparation of tissue for histological analysis and electron microscopy. In preparation for histological analysis, tissue samples from the cecum and colon were removed and placed in 10% neutral buffered formalin (pH 7). Sections (4

TABLE 1. Isolation of *S. pilosicoli* from fecal swabs taken at 2, 5, 7, and 11 days p.i. and clinical, postmortem, and histological data from newly weaned pigs experimentally infected with porcine strain *S. pilosicoli* 95/1000 (pigs A1 to A12), human strain *S. pilosicoli* WesB (pigs B1 to B12), or uninoculated culture medium (pigs C1 to C8)

S. pilosicoli strain	Diet no.[a]	Pig no.	Swab results (days p.i.)[b]					Weight gain[c]	Diarrhea	Lesions	
			2	5	7	11 (Ce)	11 (Co)			Gross	Histological[d]
95/1000	1	A1	+	+	+	+	+	2.5	+	Colitis	mc, ne, bc, sp
	1	A2	+	+	−	−	−	3.4	−	ND[e]	
	1	A3	+	−	−	−	−	3.8	−	ND	
	1	A4	+	+	+	+	+	2.4	+	Colitis	mc, ne, bc
	1	A5	−	+	−	−	−	1.5	−	ND	
	1	A6	+	−	−	−	−	2.0	−	ND	
								2.6 ± 0.8[f]			
	2	A7	−	+	−	−	−	3.5	−	ND	
	2	A8	−	−	−	−	+	3.2	−	ND	
	2	A9	+	−	+	+	+	1.7	+	Colitis	mc, m, ne, bc
	2	A10	−	−	−	−	−	1.9	−	ND	
	2	A11	+	+	+	+	+	1.6	+	Colitis	mc, ne, bc
	2	A12	−	−	−	−	+	1.4	−	ND	
								2.2 ± 0.8[f]			
WesB	1	B1	−	−	−	−	−	3.0	−	ND	
	1	B2	−	−	−	−	−	3.9	−	ND	
	1	B3	−	−	−	−	−	2.7	−	ND	
	1	B4	−	−	−	−	−	1.0	−	ND	
	1	B5	−	−	−	−	−	2.2	−	ND	
	1	B6	−	−	−	−	−	3.3	−	ND	
								2.7 ± 0.9[f]			
	2	B7	−	−	−	+[g]	+[g]	2.5	+	Colitis	mc, ne, bc
	2	B8	−	−	−	−	−	1.3	−	ND	
	2	B9	−	−	−	−	−	2.9	−	ND	
	2	B10	−	−	−	−	−	1.9	−	ND	
	2	B11	−	−	−	−	−	2.1	−	ND	
	2	B12	−	−	−	+	+	0.9	+	Colitis	mc, m, ne, bc
								1.9 ± 0.7[f]			
None (control)	1	C1–4	−	−	−	−	−	2.6 ± 0.8[h]	−	ND	
	2	C5–8	−	−	−	−	−	1.9 ± 0.3[h]	−	ND	

[a] Diet 1 was an antibiotic-free commercial pig weaner ration based on wheat and lupins. Diet 2 was unpelleted and based on cooked rice and lupins. Both diets have been shown to cause predisposition to swine dysentery (23).
[b] Ce, cecal swab; Co, colon swab; +, culture positive; −, culture negative.
[c] Weight gain is defined as the number of kilograms gained during the 11-day trial period.
[d] Mild histological lesions (mild colitis) were present in sections taken from control pigs and infected pigs that did not develop clinical signs. mc, moderate mucosal colitis; m, inflammatory changes in the muscularis layer; nc, neutrophilic exocytosis within intestinal crypts and at the luminal surface; bc, large numbers of *B. coli* cells present; sp, spirochete cells present in large numbers, attached by one end to the colonic epithelium.
[e] ND, not detected.
[f] Value is mean weight gain ± standard deviation for pigs in group A1 to A6, A7 to A12, B1 to B6, or B7 to B12.
[g] Spirochetes isolated from pig B7 could not be obtained in pure culture.
[h] Value is mean weight gain ± standard deviation for pigs in group C1 to C4 or C5 to C8. The individual weight gain data for pigs in group C are not shown.

μm) were made from paraffin-embedded tissue and were stained with hematoxylin-eosin and by the Warthin-Starry silver method. Tissue for transmission electron microscopy was immediately placed in chilled 2.5% glutaraldehyde for 24 h, postfixed in 1% aqueous osmium tetroxide for 1 h at 4°C, dehydrated in an ethanol series followed by propylene oxide, and then embedded in Epon 812 (Taab Laboratories, Reading, England). Ultrathin sections were cut on a Reichert Ultracut E ultramicrotome and mounted on carbon-coated grids. Grids were stained with freshly prepared uranyl acetate and lead citrate and examined with a Phillips 301 transmission electron microscope at 80 kV.

Isolation and characterization of spirochetes. Swabs obtained from the cecal and colonic walls at postmortem and fecal swabs obtained during the experiment were cultured as described above. Identification of spirochetes was based on morphological appearance under a phase-contrast microscope. Spirochetes that were isolated were typed by MEE as described previously (18). Swabs were also cultured aerobically on Trypticase soy blood agar and MacConkey's agar to determine if the pigs were colonized with *Salmonella* spp., hemolytic and mucoid *Escherichia coli*, or *Yersinia* spp.

RESULTS

Pathogenicity testing. Intestinal spirochetes were not isolated from the feces of any of the pigs prior to infection. Two of the pigs challenged with porcine *S. pilosicoli* 95/1000 and fed diet 1 (pigs A1 and A4) developed diarrhea on the second day p.i. Initially the feces were brown in color and had the consistency of wet cement, but they progressively became more watery. Diarrhea persisted in these two pigs for 7 days, at which time their feces began to return to a normal consistency. Two pigs that were challenged with *S. pilosicoli* 95/1000 and fed diet 2 (pigs A9 and A11) developed a similar type of diarrhea at 8 and 10 days p.i., respectively. Pigs infected with the human strain *S. pilosicoli* WesB showed no clinical signs for the first 10

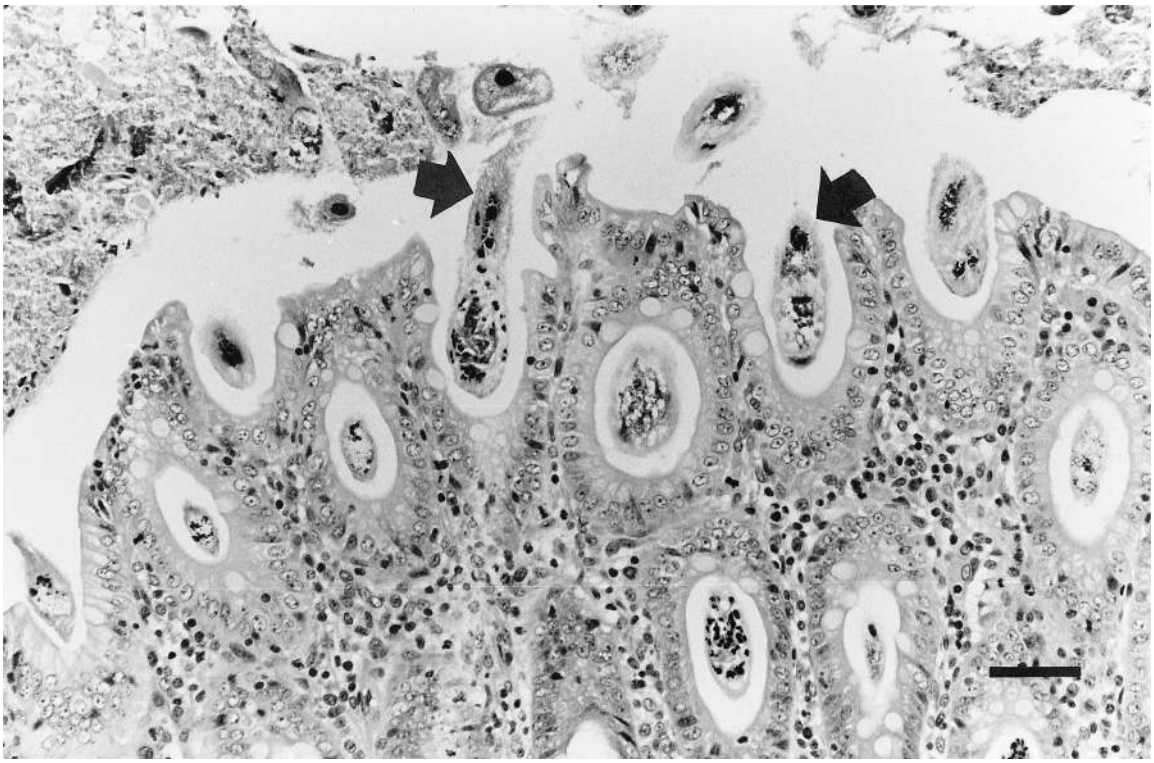

FIG. 1. Section taken from pig B12 infected with human strain *S. pilosicoli* WesB. Neutrophilic exocytosis is present within dilated intestinal crypts (arrows). The lamina propria is diffusely infiltrated with neutrophils and lymphocytes. Hematoxylin and eosin stain; bar = 37 μm.

days of the experiment; however, two of the pigs fed diet 2 developed diarrhea at 11 days p.i. (pigs B7 and B12). The feces from pig B12 were particularly watery and copious, and they had a grey-green color. All affected pigs were subjected to fecal staining of the perineum. When the growth rates of the pigs with diarrhea were compared with those of uninfected pigs or infected pigs not showing clinical signs in a one-tailed *t* test, no significant difference was found ($P < 0.05$). Similarly, no significant differences in growth rates were demonstrated when pigs fed diet 1 in groups A, B, and C were compared with pigs in the same groups that were fed diet 2 ($P < 0.05$).

S. pilosicoli 95/1000 was isolated at several time points during the experiment from all four pigs which were challenged with this strain and which developed diarrhea (Table 1). The spirochetes were also grown from cecal and colonic swabs taken at postmortem. The clinically unaffected pigs in group A were either transiently colonized between 2 and 5 days p.i. (5 of 12), positive only from colonic swabs taken at postmortem (2 of 12), or failed to become colonized (1 of 12). In contrast, the human strain *S. pilosicoli* WesB was not isolated from any pig in group B between days 2 and 11 p.i.; however, spirochetes were observed by phase-contrast microscopy in cultures taken at postmortem from the cecum and colon of pigs B7 and B12. Subculturing to obtain pure isolates was successful only for pig B12. The MEE profiles of all the spirochete isolates obtained during the experiment and at postmortem were identical to the MEE profiles of the strains originally used to infect the pigs. *Salmonella* spp., hemolytic and mucoid *E. coli*, or *Yersinia* spp. were not isolated from any pig during the course of the experiment.

Postmortem findings. Gross lesions were present in the cecum and colon of only pigs with diarrhea. When compared with clinically unaffected animals, the colons of pigs A9, A11, and B7 were increased in size, flaccid, and thin walled, and they were filled with watery, slightly mucoid contents. Localized areas of the mucosal surface were covered with small adherent nodules of digesta. When these nodules were gently removed, the underlying surface had a glistening appearance with focal areas of hyperemia. The ceca were of normal dimensions, but the mucosal surfaces were mildly inflamed. The mesenteric lymph nodes and lymphoid follicles were enlarged. Similar changes were observed in pigs A1 and A4, although the hyperemia of the luminal wall was less obvious. The most severe lesions were observed in pig B12. In addition to the above-mentioned observations, the lesions were distributed throughout the cecum and colon and a moderate to severe mucoid colitis was present in the distal colon.

Histological and ultrastructural findings. A mild subacute mucosal colitis was present in the sections taken from all unchallenged pigs and also in challenged pigs in groups A and B which did not become colonized and develop diarrhea. While the lesions varied among individual pigs, they generally were characterized by hypercellularity of the lamina propria, with an infiltrate dominated by lymphoid cells and only a few neutrophils. Mild hyperemia and edema of the lamina propria were occasionally present. The cells of the mucosal glands were normal. One or two cells of *Balantidium coli* were occasionally present in the lumen.

Histological lesions were much more severe in sections from the six pigs with diarrhea and involved both the cecum and the colon. Inflammatory changes consistent with a moderate colitis were still largely restricted to the mucosal layer, although extension into the submucosa (pigs A1, A4, A9, A11, B7, and B12) and muscularis (pigs A9 and B12) was also observed. The changes could be differentiated from those in the sections taken from the uninfected pigs, as well as the infected pigs that

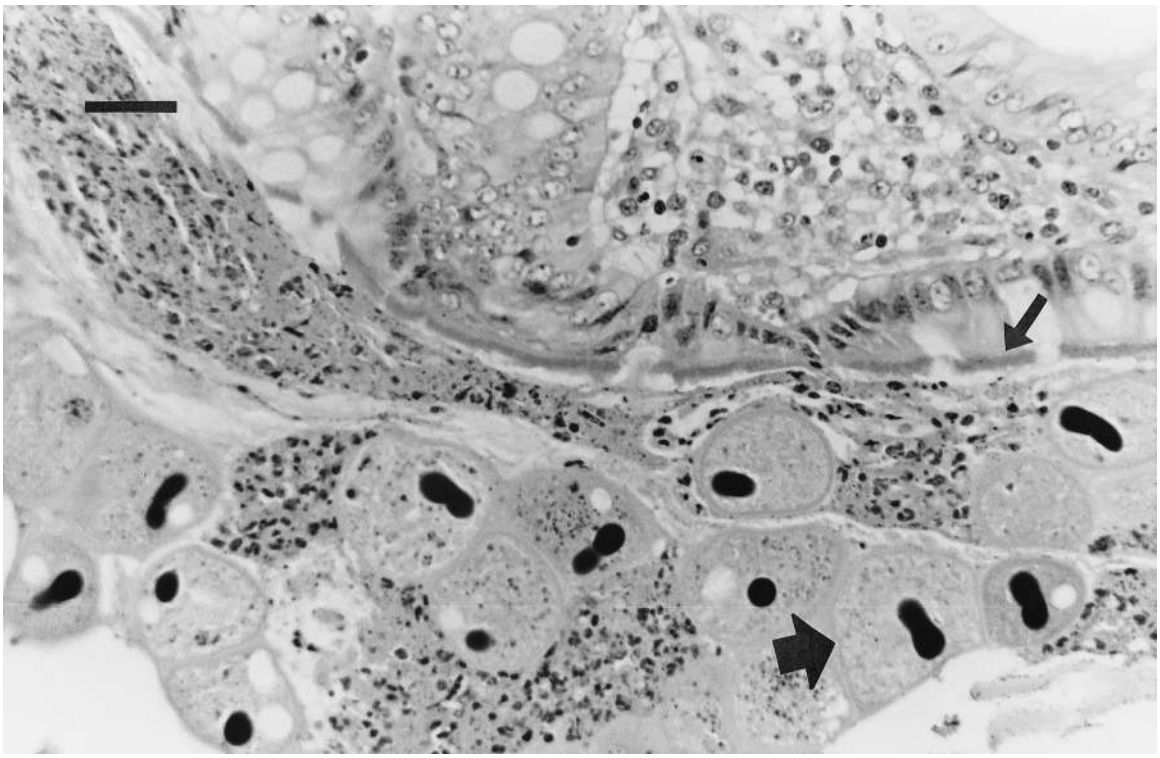

FIG. 2. Section taken from pig A1 infected with porcine strain *S. pilosicoli* 95/1000. Large numbers of *B. coli* cells are shown in close approximation to the luminal epithelium, surrounded by degenerate neutrophils (large arrow). The underlying superficial epithelium is covered by a dense layer of end-on-attached spirochetes (small arrow) Hematoxylin and eosin stain; bar = 25 μm.

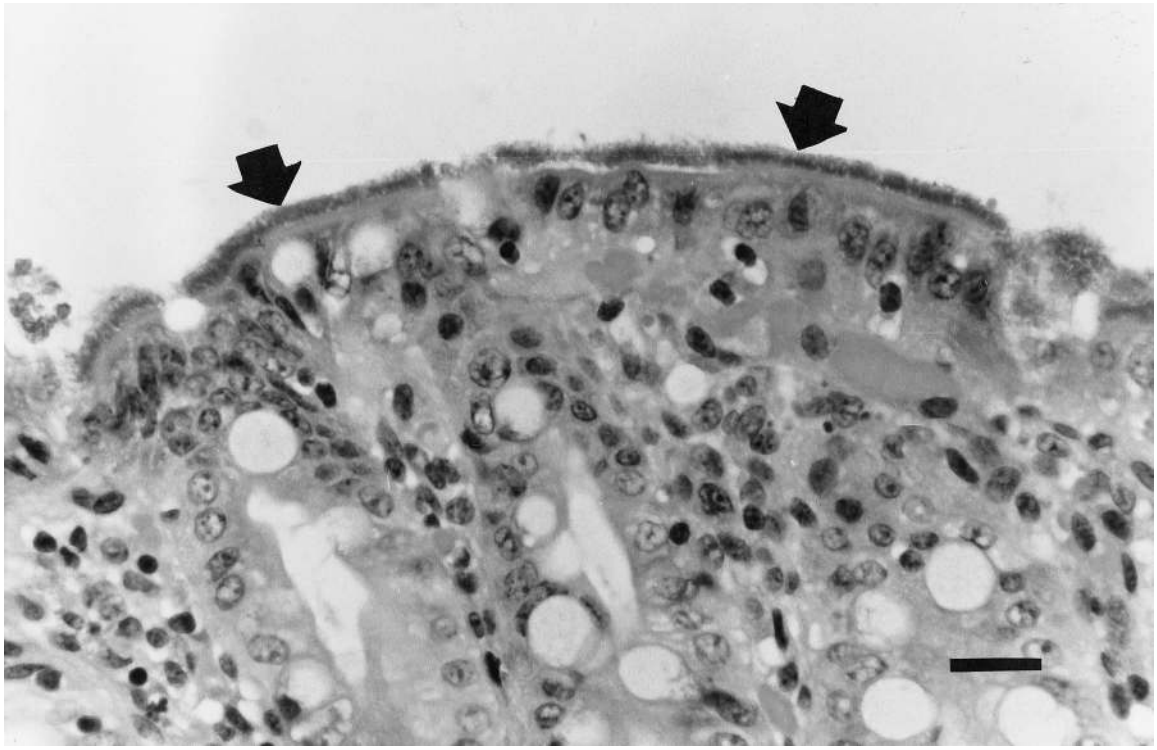

FIG. 3. Section taken from pig A1 infected with porcine strain *S. pilosicoli* 95/1000. Large numbers of spirochetes are attached by one end to the colonic epithelium (arrows). Hematoxylin and eosin stain; bar = 16 μm.

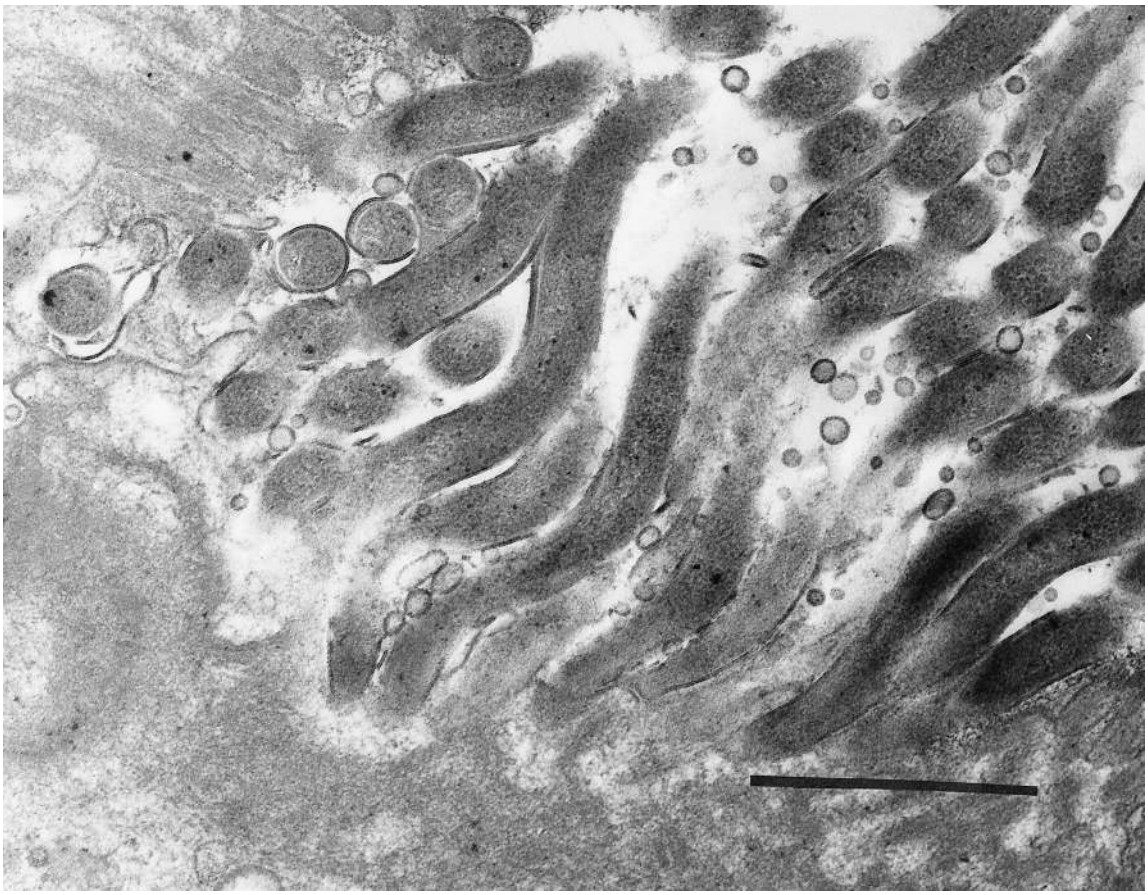

FIG. 4. Transmission electron micrograph of section taken from pig A1 infected with porcine strain *S. pilosicoli* 95/1000. Spirochete cells are invaginated into the terminal web cytoplasm of the host cells and have effaced the microvilli. Bar = 1 μm.

did not develop clinical signs, by the presence of crypt herniation into hyperplastic lymphoid follicles, and increased hypercellularity (a mixed population of both mono- and polymorphonuclear cells), and the moderate hyperemia extending from the lamina propria into the submucosa. Focal areas of neutrophilic exocytosis into dilated intestinal crypts and also at the mucosal surface were distributed throughout the sections (Fig. 1). The crypt enterocyte mitotic index was increased, and many goblet cells had discharged mucus into the crypt lumen. *B. coli* cells were found in large numbers in close approximation to the intestinal lumen and also were occasionally found within the lamina propria (Fig. 2). Sections from pig A1 were characterized by a patchy fringe of spirochetes attached by one end to mature apical enterocytes of the colonic epithelium (Fig. 3).

Silver staining showed that apart from those cells attached by one end to the colonic epithelium in sections from pig A1, the spirochetes were located within dilated intestinal crypts in close association with neutrophils, mucus, and cellular debris. Occasionally, spirochete colonization extended into the neck and mouth of the crypt, and the organisms were found in loose aggregates at the luminal surface or within the superficial layers of the lamina propria.

Transmission electron micrographs of thin sections from pig A1 showed the characteristic attachment of spirochete cells to the apical portions of superficial enterocytes (Fig. 4). Where large numbers of spirochete cells were invaginated into the terminal-web cytoplasm, the microvilli of the host cells were completely disrupted.

DISCUSSION

In this study, clinical signs and gross and histological lesions similar to those seen in naturally occurring cases of PIS were reproduced in newly weaned pigs with both a human and a porcine strain of *S. pilosicoli*. This confirmed the results of a previous study in which the disease was reproduced in 1-day-old SPF chicks with strains from both species (26). It also has extended the range of *S. pilosicoli* strains that have been shown to cause disease in experimental infections. The gross and histological lesions observed in affected pigs were similar to those recorded for both naturally occurring (6, 8, 24) and experimentally induced (1, 25) PIS. Characteristic features included mucosal typhlocolitis, the presence of neutrophilic exocytosis, excess mucus and spirochete cells within intestinal crypts, an increased crypt cell mitotic index, the presence of large numbers of *B. coli* associated with the mucosa and lamina propria, and, in one pig, the presence of spirochetes attached by one end to the colonic epithelium.

Attachment of large numbers of spirochetes by one end to the colonic or cecal epithelium is a pathognomonic feature of PIS and HIS. The human strain *S. pilosicoli* WesB and the porcine strain *S. pilosicoli* 1648 previously have been used to reproduce diarrhea and a similar one-end attachment of spirochetes to the cecal epithelium in experimentally infected 1-day-old SPF chicks (26); spirochete cells were completely absent from the intestinal crypts. On the basis of this observation, it was suggested that spirochete attachment to the mature

apical enterocytes was the major mechanism by which diarrhea was induced in the chicks through blockage of mucosal absorptive capacity. While this mechanism may operate to some extent, in the present study only one of six pigs which became colonized and developed diarrhea showed this characteristic lesion. In the remainder the spirochetes were intimately associated with neutrophilic exocytosis and mucus within dilated intestinal crypts. These results were similar to those of Taylor and coworkers (25), who found attachment of spirochetes by one end to the colonic epithelium in only one of eight pigs infected with P43/6/78^T even though six pigs had a moderate histological colitis characterized by crypt abscesses and four developed signs of diarrhea. It has been reported that in naturally infected pigs, spirochete attachment occurs only to mature cylindrical enterocytes while immature cuboidal or squamous superficial epithelium is devoid of bacterial cells (8). In the present study, infection with *S. pilosicoli* resulted in an increased crypt cell mitotic rate, and this may have resulted in a more immature epithelium and, consequently, less end-on attachment. However, the absence of one-end spirochete attachment to the epithelium in the majority of affected pigs could be the result of a number of factors, including the genotype of the pig, if attachment is through specific surface receptors on enterocytes.

Pigs fed either diet became colonized with *S. pilosicoli* and developed diarrhea. Both diets contain large amounts of nonstarch polysaccharides and oligosaccharides, which are not well absorbed in the small intestine and pass largely undigested to the large intestine (23). Subsequent increased bacterial fermentation in the large intestine is thought to create a favorable environment for *S. hyodysenteriae*, and this may also be the case for *S. pilosicoli*. The mild subclinical colitis that was noticed in the control animals fed either diet could have been caused by products of the increased bacterial fermentation in the large intestine. Similar mild histological changes previously have been reported in the colon of young, apparently healthy pigs (19). A diet containing large amounts of fermentable ingredients (wheat and soy extract) has also been shown to promote attaching and effacing lesions in the large intestine of pigs associated with enteropathogenic *E. coli* strains (20).

Only 4 of 12 pigs challenged with the porcine *S. pilosicoli* strain and 2 of 12 challenged with the human *S. pilosicoli* strain developed clinical signs and significant histological changes. Depression of growth in the affected pigs was not significant at the termination of the experiment; however, at this point in time, four of the pigs had only just begun to show clinical signs. Two other pigs infected with the porcine strain were culture positive at postmortem. It seems likely that more pigs would have shown colonization and/or disease and reduction in weight gain if the experiment had not been terminated after 11 days, as the incubation period for PIS has been reported to range from 3 to 20 days (25). The results suggest that the human strain was not as well adapted to colonization of the porcine large intestine as the porcine strain. Nevertheless, the bacterium was shown to cross host species boundaries and induce disease, and it now appears increasingly likely that animal strains of *S. pilosicoli* could colonize human beings under suitable circumstances.

In this experiment, the role of coinfection with *B. coli* as a precipitating factor in PIS was unclear. *B. coli* is considered to be a nonpathogenic commensal organism; however, it does invade and multiply in damaged epithelium, and it has been associated with a number of porcine colonic diseases, including salmonellosis and swine dysentery (2). Interestingly, a case of intestinal spirochetosis recorded in a puppy was associated with giardiasis (5), and in a prevalence study conducted among Aboriginal children, fecal samples that were positive for *S. pilosicoli* were also positive for *Giardia duodenalis* (16). A possible interaction between *S. pilosicoli* and gastrointestinal protozoa in inducing disease requires further investigation.

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