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Pathogenicity of Three Strains of *Serpulina pilosicoli* in Pigs with a Naturally Acquired Intestinal Flora

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***Serpulina pilosicoli* is an anaerobic spirochete which has been isolated from the colons of pigs with enteric disease. The clinical and pathologic features of experimental infections of conventional pigs (born by normal farrowing with a naturally acquired intestinal flora) with three strains of *S. pilosicoli* were determined in order to confirm the enteropathogenicity of this species. Strains were derived from the colons of British pigs with colitis and passaged 8 to 10 times during expansion and purification in vitro. Eighteen ten-week-old Large White-Landrace cross pigs were each inoculated once orally with 0.7×10^9 to 1.6×10^9 of one of three strains of *S. pilosicoli*. Six pigs were challenged with each strain. Control pigs were dosed with uninfected broth medium or with 1.8×10^7 cells of the nonpathogenic *Serpulina innocens*. Eight pigs (two to four per *S. pilosicoli* challenge group) developed soft or diarrheic feces (fecal dry matter < 24%) between 3 and 8 days after challenge, which persisted for 7 to 8 days or until necropsy at 14 days after challenge. Average weight gains in two of the three groups challenged with *S. pilosicoli* were significantly less than controls. The feed conversion ratios of all the groups challenged with *S. pilosicoli* were impaired compared to controls. The mean values for daily liveweight gain (and feed conversion ratio) for the three groups challenged with *S. pilosicoli* were 0.799 (2.13), 0.783 (2.05), and 0.844 kg (2.10), respectively, while that of the uninoculated controls was 0.944 kg (1.70). Gross lesions with slight mucosal thickening, congestion, and multifocal erosions were evident in seven of eight diarrheic pigs. The relative weights of the large intestines of pigs challenged with *S. pilosicoli* were significantly less than controls. Histologic lesions with an increase in mucosal height, infiltration of the lamina propria with mononuclear cells, mucosal erosion with mixed inflammatory cell infiltration, and goblet cell hyperplasia in colonic glands were evident in 15 of the 18 challenged pigs. *S. pilosicoli* was recovered on bacterial culture of the colon from all except one of the pigs with these histologic lesions. *Serpulina* sp. was clearly visible within the colonic glands of these affected pigs in silver-stained sections of the gut. Clinical and pathologic findings in control pigs were unremarkable, with no diarrhea or colonic lesions evident. The results provide further evidence that *S. pilosicoli* is a specific enteric pathogen for conventional pigs. It is capable of colonizing the large intestine and causing mucosal damage, which although mild is sufficient to result in significant adverse effects on growth.**

There are three species of *Serpulina* spirochetes currently known to occur in the intestinal tract of the pig. *Serpulina hyodysenteriae* is strongly hemolytic when cultured on blood agar, possesses three known hemolysins, and causes mucohemorrhagic typhlocolitis when inoculated orally into conventional pigs (born by normal farrowing with a naturally acquired intestinal flora) (8, 9, 20). *Serpulina innocens* is weakly hemolytic on blood agar and has failed to cause visible lesions when inoculated orally into conventional or gnotobiotic pigs (12, 18).

Serpulina pilosicoli, previously known as group IV *Serpulina* sp. (4, 27), has been linked with the presence of diarrhea in field investigations of enteric disease in pigs (3, 6, 11, 13, 23). The disease associated with this infection has variably been termed spirochetal diarrhea (22, 23), porcine colonic spirochetosis (3, 6), and porcine intestinal spirochetosis (26, 27). The disease affects weaned pigs approximately 7 to 20 weeks of age, including those of high health status such as minimal disease and specific pathogen-free herds which are free of *S. hyodysenteriae* infections. Typically, field cases show moderate, nonhemorrhagic diarrhea and reduced growth rates (3, 4, 23). Isolates from field cases have frequently included *S. pilosicoli*, a weakly beta-hemolytic anaerobic spirochete which is characterized biochemically by being positive for hippurate hydrolysis and α-galactosidase activity but negative for indole production, α-glucosidase, and β-glucosidase activities (4).

There have been few reports of experimental reproduction of clinical diarrhea following oral inoculation of conventional pigs with weakly beta-hemolytic spirochetes (1, 23, 26). In the first study, strain P43/6/78^T (27), which was isolated from a British pig, was found to cause colitis in six of eight challenged pigs, with spirochetes resembling P43/6/78 reisolated from their colons (23). More recently, experimental challenge of recently weaned piglets with an Australian strain of porcine origin and one of human origin was found to cause diarrhea and colitis in 4 of 12 and 2 of 12 pigs, respectively (26). A further feature of some pigs in the experimental studies and in some field cases of spirochetal colitis was the presence of numerous spirochetes attaching "end on" to the surface of the colonic epithelium (3, 10, 23, 26). In further studies with strain P43/6/78^T, colonic lesions were evident in inoculated gnotobiotic piglets (17, 18). However, in conventional and conventionalized British pigs, P43/6/78^T and two phenotypically similar strains failed to induce diarrhea or significant lesions in any of the challenged animals (17). (Conventionalized pigs are hysterotomy derived and artificially colonized with an enteric flora that is free from porcine enteric pathogens.)

The reasons for the failure of some oral inoculation experiments are not clear but could include attenuation of the bac-

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TABLE 1. Experimental protocol

Group	No. of pigs	Strain used for challenge	Dose per pig
1[a]	9		
2	3	*S. innocens* P121/6/93	1.8×10^7
3	6	*S. pilosicoli* P51/6/93	1.6×10^9
4	6	*S. pilosicoli* P99/1/93	1.3×10^9
5	6	*S. pilosicoli* P100/6/93	7×10^8

[a] Group 1 was the control group.

terial strains during in vitro culture, variation in pathogenic potential between strains, and differences in host response or particular cofactors, for example, differences in dietary composition. In view of the increasing number of field problems associated with *S. pilosicoli* in the United Kingdom in recent years and the variable results of previous pathogenicity studies, we decided to investigate the enteropathogenicity of several strains of *S. pilosicoli* isolated from recent naturally occurring cases of colitis in British pigs.

(An abstract summarizing preliminary findings of part of this research has been published previously [25].)

MATERIALS AND METHODS

Bacterial strains. Three strains of *S. pilosicoli*, P51/6/93 (NCTC 12874), P99/1/93 (NCTC 12875), and P100/6/93, and one strain of *S. innocens*, P121/6/93, were isolated from fresh colonic mucosal scrapings of separate pigs submitted for necropsy during natural outbreaks of enteric disease on four commercial pig farms. Pigs had been suffering from chronic nonhemorrhagic diarrhea and had macroscopic and histologic lesions of colitis, with apparent colonization of colonic glands by large spirochetes. Initially, the scrapings were cultured on sheep blood agar supplemented with spectinomycin (400 μg/ml), colistin (25 μg/ml), vancomycin (25 μg/ml), and ribonucleic acid (10 μg/ml) at 37°C in an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Subcultures on blood agar were tested for purity by Gram staining of dried smears. The isolates were classified on the basis of degree of hemolysis and biochemical reactions as previously described (4) and by a PCR test specific for this group of organisms (5). Samples of each strain were dried onto copper grids, stained with 2% phosphotungstic acid, and examined under a transmission electron microscope (Philips EM400) for cell size, morphology, and the number of axial flagellae. Each strain of *S. pilosicoli* listed above had 5 axial flagellae at each end, while the *S. innocens* isolate had 12, consistent with previous descriptions (23, 27). Isolates were stored in nutrient broth with 10% rabbit serum at −70°C until required.

Oral inocula. Pure cultures of each strain were expanded by anaerobic culture on blood agar plates for 72 h and then harvested into 20 ml of brain heart infusion broth containing 5% normal rabbit serum and cultured anaerobically for a further 48 h. The broth cultures were transferred into flasks containing 200 ml of the above broth medium and cultured for a further 48 h. The broth cultures were checked for purity by microscopic examination of Gram-stained smears and by aerobic and anaerobic cultures on blood agar plates. Test cultures used for inoculation had been passaged between 8 and 10 times on laboratory medium. A single challenge dose of 10 ml of broth culture was given orally to each pig via a syringe attached to an intraesophageal tube. Samples of each inoculum were cultured anaerobically on blood agar incorporating the Miles-Misra method of culture dilutions to estimate bacterial numbers (16).

Experimental design. Thirty Large White-Landrace cross pigs (7 weeks old) which had been weaned at 3 weeks of age were allocated into five groups on a randomly stratified basis. The pigs were all males and weighed between 15 and 16 kg. They were numbered individually and housed in groups of three per pen for a further 19-day acclimatization period prior to oral challenge. The pens were solid floored with solid partitions between groups. The pens were lightly bedded with wood shavings in the lying areas, and the floors were scraped clean twice daily. The pigs were fed a commercial pelleted diet (wheat, barley, soya bean meal, fishmeal, fat, vitamin, and mineral balancer). The diet contained 20.5% protein, had added copper sulfate (175 mg/kg), and was fed ad libitum throughout the study with single-space hopper feeders. The weight of feed consumed by each group during the challenge period was recorded. Water was provided via nipple drinkers.

The species, strain, and number of bacteria in each challenge inoculum are shown in Table 1. Control pigs (Group 1) were dosed orally with 10 ml of the uninfected broth medium. The challenge dose of *S. innocens* was 1.8×10^7 organisms, while the doses for the three groups challenged with *S. pilosicoli* ranged from 7.0×10^8 to 1.6×10^9 organisms. Pigs were inspected daily for any evidence of diarrhea or clinical illness, and body temperatures were recorded. The fecal consistency for each pig was scored on a scale of 0 (normal) to ++++ (watery diarrhea) daily, and the percent fecal dry matter was determined twice weekly by weighing, drying, and reweighing individual samples. Pigs were weighed weekly, and blood was sampled for full hematological examination twice weekly. Hematological examination comprised erythrocyte count, hemoglobin concentration, mean cell volume, mean hemoglobin concentration, leukocyte count, and differential leukocyte count.

Bacteriology. Fecal samples from each of the pigs were cultured twice weekly before and after challenge for the presence of certain bacteria as follows. Spirochetes were grown anaerobically at 37°C for up to 7 days on sheep blood agar supplemented with spectinomycin (400 μg/ml), colistin (25 μg/ml), vancomycin (25 μg/ml), and ribonucleic acid (10 μg/ml) and speciated by morphology and biochemical reactions (4). *Yersinia* spp. were detected by aerobic incubation of *Yersinia* selective agar (Oxoid) at 30°C for 48 h. The identities of sample colonies were confirmed with the API 20E system (Biomerieux). Salmonellae were cultured by inoculation of Rappaport-Vasiliadis broth and aerobic incubation at 37°C for 24 h, followed by subculture on brilliant green agar and incubation for 24 to 48 h.

Necropsy. The pigs were submitted for necropsy at 14 or 15 days after challenge. The pigs were killed by captive-bolt stunning and exsanguination, and the intestines were removed as quickly as possible. The weights of the entire small and large intestines with contents and that of the separated large intestine with contents were recorded in order to calculate the ratio of large intestine to entire intestine. The intestines were opened, and samples taken from the duodenum, jejunum, ileum (comprising six levels of the small intestine), cecum, proximal colon, mid-spiral colon, distal colon, and rectum were immersed in 10% buffered formalin. The sample collection was completed within 3 min of death. The intestines were then examined closely, and any macroscopic abnormalities were noted. A complete postmortem examination was performed on each pig to look for any other lesions which may have affected growth rate. The pigs were presented for necropsy in a random sequence, with the animal number and group identity provided to the pathologist only on completion of the necropsy and recording of findings.

Scrapings of the fresh mucosa of the proximal and distal colons were cultured as described above for feces, and the colonic contents were examined microscopically for parasitic infection. The fixed samples of intestine were processed routinely for histologic examination, and separate sections were stained with hematoxylin and eosin and Warthin-Starry silver impregnation (21). The his-

TABLE 2. Number of pigs with soft or diarrheic feces and percent fecal dry matter results

Group	Prechallenge status (day 0)		Results[c]							
			Day 3		Day 7		Day 10		Day 14	
	D[a]	% FDM[b]	D	% FDM	D	% FDM	D	% FDM	D	% FDM
1	0/9	29.5 ± 2.0 (27.0–32.9)	0/9	29.2 ± 1.8 (27.0–32.0)	0/9	29.2 ± 2.1 (26.7–32.4)	0/9	29.9 ± 1.9 (27.4–33.6)	0/9	29.8 ± 2.5 (27.0–35.2)
2	0/3	29.8 ± 3.5 (27.4–33.8)	0/3	27.1 ± 0.2* (26.9–27.3)	0/3	27.8 ± 1.2 (27.1–29.2)	0/3	28.0 ± 1.8 (26.4–29.9)	0/3	28.2 ± 1.7 (26.4–29.7)
3	0/6	30.0 ± 1.6 (28.3–32.6)	0/6	27.5 ± 2.2 (25.5–31.3)	2/6	26.6 ± 4.1 (21.4–31.5)	2/6	27.1 ± 2.9 (23.2–30.9)	1/6	26.6 ± 2.3* (23.0–29.8)
4	0/6	29.5 ± 2.0 (26.5–31.8)	1/6	26.3 ± 1.9* (23.3–28.8)	4/6	24.2 ± 3.7* (19.0–29.3)	2/6	24.8 ± 3.4** (18.1–27.8)	4/6	23.9 ± 3.6** (20.3–30.1)
5	0/6	30.5 ± 1.4 (28.1–32.2)	0/6	29.3 ± 1.0 (28.0–30.7)	2/6	27.1 ± 4.0 (21.9–31.2)	2/6	26.9 ± 2.4* (23.6–29.5)	1/6	26.6 ± 2.5* (22.2–29.4)

[a] D, number of pigs with soft or diarrheic feces.

[b] % FDM, percent fecal dry matter. The fecal dry matter weight is expressed as a percentage of the fresh feces weight. Results are expressed as means ± SD with the range of values for individual pigs given in parentheses.

[c] Results are for the indicated days postchallenge. Statistical difference from controls (group 1) by the two-sample Student's *t* test is indicated as follows: *, $P < 0.05$; **, $P < 0.01$.

TABLE 3. Average daily liveweight gains and postchallenge feed conversion ratios

Group	ADG (kg)[a]		Postchallenge FCR[b]
	Prechallenge	Postchallenge	
1	0.733 ± 0.101	0.944 ± 0.130	1.70
2	0.700 ± 0.129	0.860 ± 0.066	1.83
3	0.741 ± 0.081	0.799 ± 0.121*	2.13
4	0.729 ± 0.043	0.783 ± 0.124*	2.05
5	0.687 ± 0.046	0.844 ± 0.185	2.10

[a] ADG, average daily liveweight gain (means ± SD). Prechallenge averages determined over 19 days, and postchallenge averages determined over 14 days. *, statistically different from controls (group 1) by two-sample Student's *t* test ($P < 0.05$).

[b] FCR, feed conversion ratio. Values derived from feed consumption of group over 14 days postchallenge (in kilograms) divided by the total weight gain of pigs in group (in kilograms).

topathological sections were coded and examined blind in order to overcome experimental bias.

Statistical analyses. The average daily liveweight gain before and after challenge, percent fecal dry matter, and hematologic values for each of groups 2, 3, 4, and 5 were compared with controls (group 1) at each sampling by means of the two-sample Student's *t* test (Microsoft Excel 4.0). In addition, the leukocyte counts (means ± standard deviations [SD]) of a subset of 8 clinically affected challenged pigs derived from groups 3, 4, and 5 were compared with those of the 10 asymptomatic challenged pigs in the same groups as well as with the unchallenged controls (group 1) at each sampling, again by using the two-sample Student's *t* test.

RESULTS

Clinical findings. All pigs in groups 1 and 2 (controls and *S. innocens* challenge, respectively) remained healthy and nondiarrheic throughout the study (Table 2). All pigs in groups 3, 4, and 5 (*S. pilosicoli* challenge) were healthy and had similar average daily weight gains to those in groups 1 and 2 prior to the challenge. Two pigs in each of groups 3 and 5 and four pigs in group 4 developed soft to diarrheic feces (score, ++ or +++) after challenge. Abnormal consistencies were first noticed between 4 and 8 days after challenge and persisted for at least 7 days or until euthanasia (Table 2). The average daily weight gains and feed conversion ratios of pigs in groups 1 and 2 were markedly better than those in groups 3, 4, and 5 after challenge (Table 3). The body temperatures of all pigs remained within the normal range (39 ± 0.5°C) throughout the trial. There were no significant differences in hematologic parameters between any of the challenged groups and the controls. However, at 10 to 14 days after challenge, six of the eight clinically affected pigs developed mild leukocytosis. The respective mean (± SD) leukocyte counts for the 8 affected pigs at 10 and 14 days post-infection were $(27.6 \pm 8.9) \times 10^9$ and $(33.8 \pm 7.3) \times 10^9$, whereas the values for the 10 asymptomatic pigs on these days were $(19.3 \pm 1.8) \times 10^9$ and $(19.6 \pm 3.4) \times 10^9$, respectively, and those for the unchallenged control group were $(18.3 \pm 5.5) \times 10^9$ and $(20.6 \pm 3.9) \times 10^9$, respectively. The differences between the values for the symptomatic and asymptomatic challenged pigs were significant at both times ($P < 0.05$). Likewise the differences between the values for the symptomatic pigs and the unchallenged control pigs were significant at both times ($P < 0.05$).

Bacteriology and necropsy findings. *S. pilosicoli* was cultured from 14 pigs in groups 3, 4, and 5 following but not before challenge. *S. innocens* was isolated from six pigs (two pigs in each of groups 1, 3, and 5) both before and after challenge, indicating a natural infection in those pigs. *S. innocens* was cultured from two of the three pigs in group 2 but only after challenge with *S. innocens* (Table 4). No salmonellae or *Yersinia* spp. were isolated from any fecal or colonic sample.

Gross changes of slight thickening of the colonic mucosa, congestion, and multifocal areas of mucosal erosion were evident in seven of the eight diarrheic pigs in groups 3, 4, and 5. No other gross lesions were detected in any pig. Examination of histologic sections indicated an increase in mucosal height of the large intestine accompanied by an increase in crypt depth and moderate infiltration of the mucosa by mononuclear cells in most pigs in groups 3, 4 and 5 (Table 4 and Fig. 1a). Mononuclear cell infiltration was present in the submucosa. Pigs which had been diarrheic showed multifocal areas of mucosal erosion, subepithelial congestion, edema, and mixed inflammatory cell infiltration at the erosion sites (Fig. 2a). There was an apparent increase in the number of goblet cells in the glands and surface epithelium of the colonic mucosa. Many glands appeared dilated with an apparent increase in the quantity of goblet cell mucin in the crypt lumens of these affected pigs. Some crypts showed degeneration and inflammation. Silver-stained sections of the large intestine showed many large spirochetes along the surface epithelium and in the colonic glands in 15 of 18 pigs challenged with *S. pilosicoli* (Fig. 3 and Table 4). Spirochetes were present in or on crypt goblet cells (Fig. 4) and were occasionally seen in the lamina propria. There was no visible accumulation of spirochetes attached end on onto the epithelial surface of any pig, despite careful examination of silver-stained sections. In summary, in the subset

TABLE 4. Necropsy findings and bacteriology

Group	No. of pigs	No. of pigs with:					LI/TI ratio[d]
		Gross lesions of colitis[a]	Histopathological lesions of colitis[b]	Spirochete colonization[c]			
				Microscopic analysis	Culture		
					S. pilosicoli	*S. innocens*	
1	9	0	0	0	0	2	0.47 ± 0.02 (0.44–0.51)
2	3	0	0	2	0	2	0.47 ± 0.03 (0.44–0.50)
3	6	2	6	6	5	2	0.43 ± 0.04* (0.38–0.48)
4	6	3	5	5	5	0	0.40 ± 0.04* (0.35–0.45)
5	6	2	4	4	4	2	0.45 ± 0.04 (0.37–0.49)

[a] Presence of mucosal thickening, erosions, and inflammation. There were no significant lesions elsewhere in any pig.

[b] See text for details.

[c] Viable spirochetes present in the colon. A positive determination by microscopic analysis indicated that many spirochetes were visible in crypts of the colon. Colonization was also determined by the culturing of spirochetes from colonic mucosa.

[d] Ratio of large intestine weight (LI) to entire intestine weight (TI). Results are means ± SD with the range of values for individual pigs given in parentheses. *, statistical difference from controls (group 1) by the two-sample Student's *t* test ($P < 0.05$).

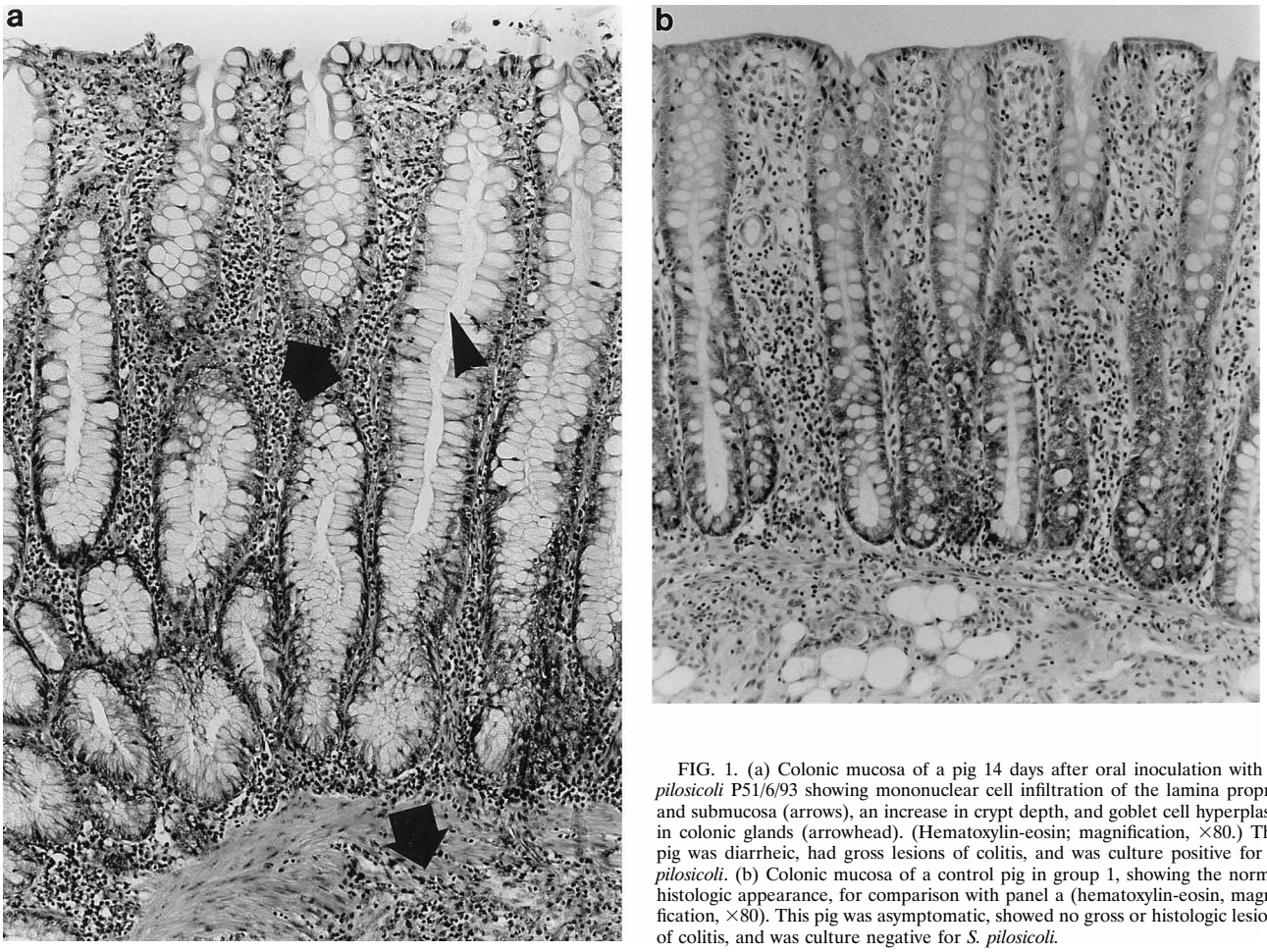

FIG. 1. (a) Colonic mucosa of a pig 14 days after oral inoculation with *S. pilosicoli* P51/6/93 showing mononuclear cell infiltration of the lamina propria and submucosa (arrows), an increase in crypt depth, and goblet cell hyperplasia in colonic glands (arrowhead). (Hematoxylin-eosin; magnification, ×80.) This pig was diarrheic, had gross lesions of colitis, and was culture positive for *S. pilosicoli*. (b) Colonic mucosa of a control pig in group 1, showing the normal histologic appearance, for comparison with panel a (hematoxylin-eosin, magnification, ×80). This pig was asymptomatic, showed no gross or histologic lesions of colitis, and was culture negative for *S. pilosicoli.*

of eight diarrheic pigs, seven showed gross lesions of colitis, all had histological evidence of colitis, and all were culture positive for *S. pilosicoli*. Large spirochetes were not observed in any of the pigs in groups 1 and 2. The histological appearance of the colonic mucosa was within normal limits for all pigs in groups 1 and 2.

Moderate numbers of smaller spirochetes resembling *S. innocens* were present in the colonic glands of two of three pigs in group 2, both of which yielded *S. innocens* at necropsy. These bacteria were approximately half the length of those present in the *S. pilosicoli*-challenged pigs. Occasional organisms resembling *S. innocens* were seen in the pigs in groups 1, 3, and 5, which had been culture positive. Preinfection with *S. innocens* did not prevent colonization by *S. pilosicoli* in three of four pigs.

The intestinal weight ratios are shown in Table 4. These indicate a significant reduction in the weight of the large intestine and its contents in *S. pilosicoli*-challenged pigs in groups 3 and 4 compared to controls.

DISCUSSION

This study confirms the ability of orally inoculated *S. pilosicoli* strains to colonize the colon and to cause diarrhea and colitis in conventional pigs. Therefore, culture of *S. pilosicoli* from naturally occurring colitis in pigs is likely to be a significant event, particularly in the absence of any other enteric pathogens. This disease has emerged as a significant problem in intensive pig-farming systems throughout the world (3, 4, 6, 11, 22, 27). The clear disturbances in weight gains and feed conversion ratios in affected pigs in this study, and a previous study (23), demonstrate the possible losses of production which could occur in field outbreaks of this disease. Combined infection with other forms of enteric disease, particularly *Yersinia pseudotuberculosis* and proliferative enteropathy due to *Lawsonia intracellularis* is often seen in field cases in the United Kingdom (24), and this is likely to have further adverse effects on productivity.

The incubation period of between 4 and 9 days in the clinically affected pigs was similar to that reported for a previous study (5 to 8 days) (23), whereas another study indicated incubation periods as short as 2 days (26). In the present study, slight leukocytosis was recorded in the symptomatic pigs at 10 and 14 days postinfection, suggesting a mild systemic reaction. However, there was no evidence of a febrile response in any of the challenged pigs.

The colonic lesions in pigs with *S. pilosicoli* infection are relatively mild compared with the more severe hemorrhagic typhlocolitis associated with *S. hyodysenteriae*. The mechanism causing the clinical diarrhea observed in some pigs following *S. pilosicoli* challenge is uncertain. Studies of pigs experimentally infected with *S. hyodysenteriae* have shown that dehydration is the result of absorptive failure rather than increased mucosal permeability or secretory loss from the damaged mucosa (2). Although clinical dehydration was not evident in pigs chal-

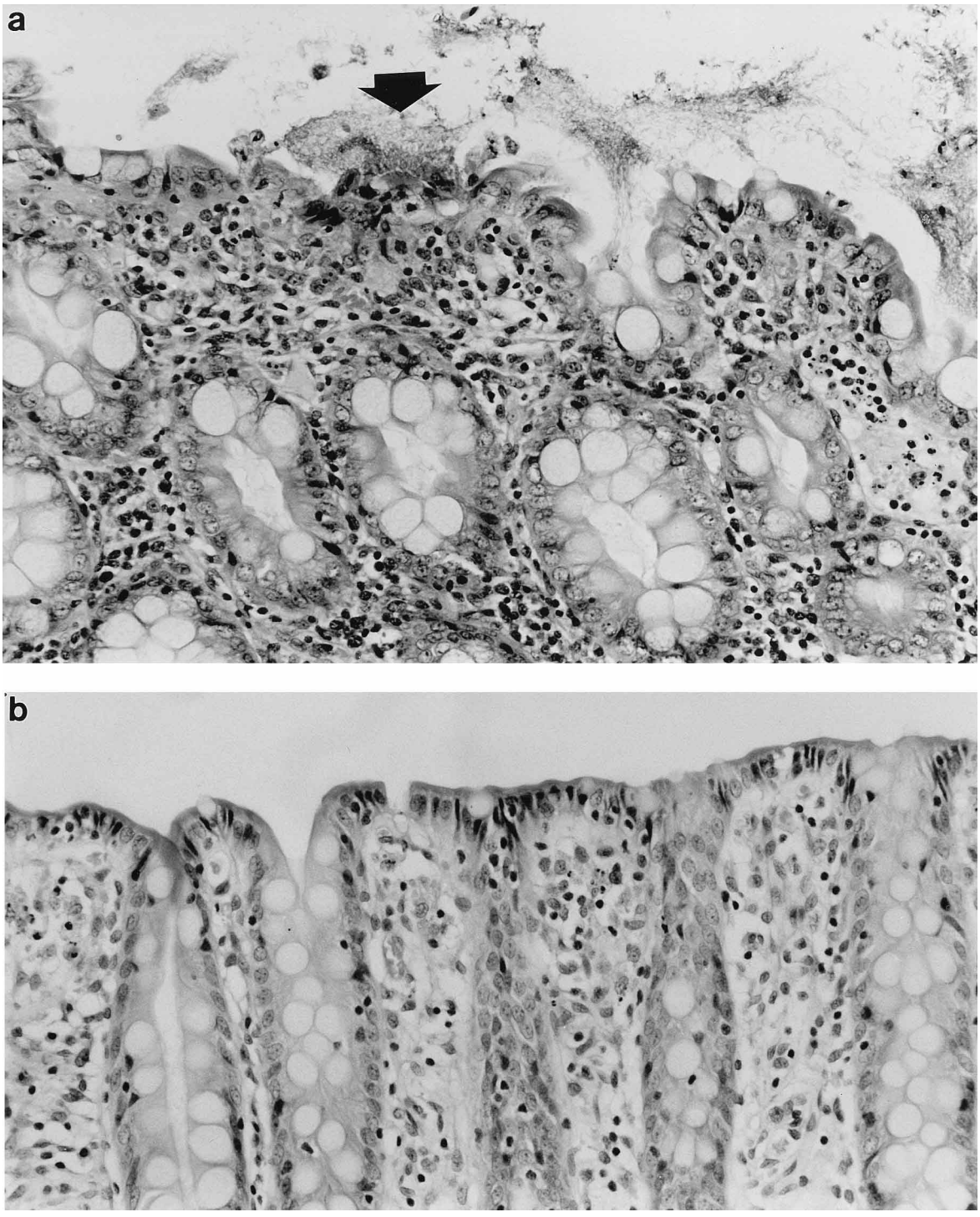

FIG. 2. (a) Superficial colonic mucosa of a pig 14 days after oral inoculation with *S. pilosicoli* P51/6/93 showing erosion and inflammation of the mucosa (arrow) associated with bacterial colonization. (Hematoxylin-eosin; magnification, ×200.) This pig was diarrheic, had gross lesions of colitis, and was culture positive for *S. pilosicoli*. (b) Superficial colonic mucosa from a control pig in group 1, showing normal histologic appearance, for comparison with panel a (hematoxylin-eosin; magnification, ×200). This pig was asymptomatic, showed no gross or histologic lesions of colitis, and was culture negative for *S. pilosicoli.*

lenged with *S. pilosicoli*, it seems likely that diarrhea might also be the result of failure in absorptive mechanisms, albeit in a milder form than in swine dysentery. Reduced fluid absorption is likely to give rise to shorter hindgut retention times and, consequently, a reduction in volatile fatty acid production and absorption. In growing pigs on cereal diets, volatile fatty acid production supplies 25 to 30% of the animal's maintenance energy requirements (2, 19). Thus, an alteration in this process could result in reduced feed conversion, as seen in this study. Some dietary factors have been shown to cause diarrhea and

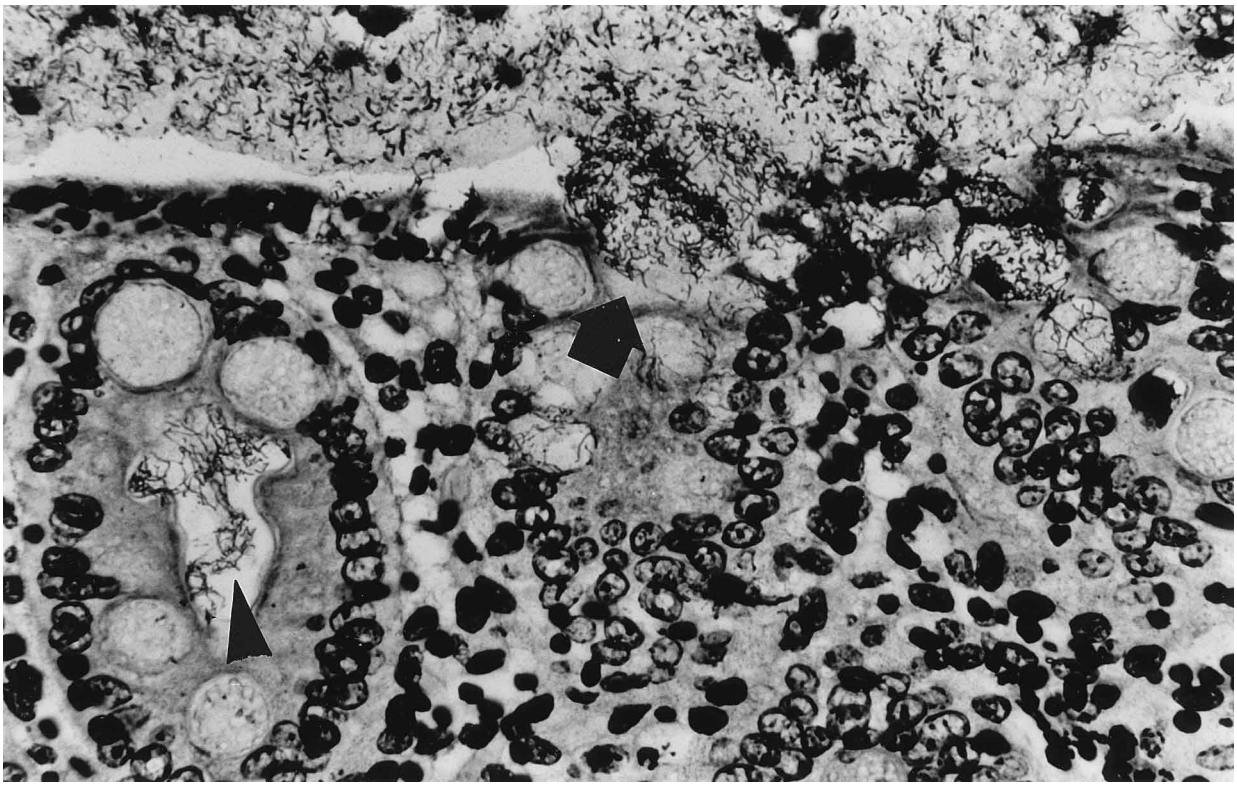

FIG. 3. Colonic mucosa of a pig 14 days after oral inoculation with *S. pilosicoli* P99/1/93 showing heavy colonization with large spirochetes in the mouth (arrow) and the lumen of glands (arrowhead). (Warthin-Starry; magnification, ×400.) This pig was diarrheic, had gross and histologic lesions of colitis, and was culture positive for *S. pilosicoli.*

colitis directly (11, 17). However, the lack of diarrhea or lesions in pigs that had been dosed with uninoculated broth or *S. innocens* indicates that the diet and husbandry of the challenged pigs were not inherently pathogenic.

As the dose of *S. innocens* used in this study was lower than that in the *S. pilosicoli*-challenged groups, the results are not strictly comparable. It was not possible to achieve identical challenge doses for each of the experimental groups as log-phase cultures were used for challenge and viable counts were only established retrospectively. However, it was decided to include these results for completeness and for future reference. Similarly, the doses of *S. pilosicoli* varied slightly between groups. These differences did not appear to affect the development of disease in general. However, minor differences in presentation could be due to the different sizes of inocula. Recovery of *S. innocens* from groups 1, 3, and 5 prior to challenge indicates that this organism was present naturally in some of the pigs. The presence of *S. innocens* did not appear to either enhance the effect of *S. pilosicoli* challenge or protect against it. No fecal blood was evident in challenged pigs in this study, and it is rarely seen in field cases of colitis associated with *S. pilosicoli*. While *S. hyodysenteriae* possesses hemolysins and causes a muco-hemorrhagic colitis (15), it is still not clear if and how the two phenomena are related. The presence of hemolysin in *S. pilosicoli* has not been determined as yet so its potential as a virulence determinant is unknown.

The main finding of this study, that British strains of *S. pilosicoli* can be pathogenic, agrees with a previous challenge study using another British strain (P43/6/78^T) (23) and a recent study using two Australian isolates (26). However, other challenge studies in conventional pigs using the type strain and two phenotypically similar strains have failed to demonstrate diarrhea or noticeable lesions (17). There are several possible factors which may explain the disparate results. There may be considerable intrinsic variation in the phenotype of *S. pilosicoli*, with some strains being pathogenic and others less so. Different electrophoretic types of *S. pilosicoli* have been demonstrated by multilocus enzyme electrophoresis (14), and it would be useful to compare the electrophoretic types of the strains which have been found to be pathogenic in conventional pigs. Furthermore, there may have been differences in our preparation of cultures derived from field cases of intestinal disease prior to use as challenge inocula. We attempted to use isolates with a minimal number of in vitro passages to minimize the potential for attenuation of virulence. Also we attempted to use adequate bacterial numbers in the challenge inocula to minimize potential underdosing.

Diet has been suggested as being an important cofactor in the development of porcine spirochetal infections (17), though the use of two different diets in the Australian study did not appear to have a significant bearing on susceptibility to infection (26). It remains uncertain whether the wheat-barley diet used in the present study was an important contributory factor but there is a need for a better understanding of the role of diet in the pathogenesis of porcine *Serpulina* spp. infections. Preinfection of conventional pigs with *S. innocens* has been suggested as a possible reason for failure of some challenge experiments, through the conferring of a degree of local mucosal immunity (17). In this study, successful colonization of *S. pilosicoli* in three of four pigs which were known to be carrying *S. innocens* from 14 days prior to challenge suggests that this is not the case. However, the exact duration of *S. innocens* infec-

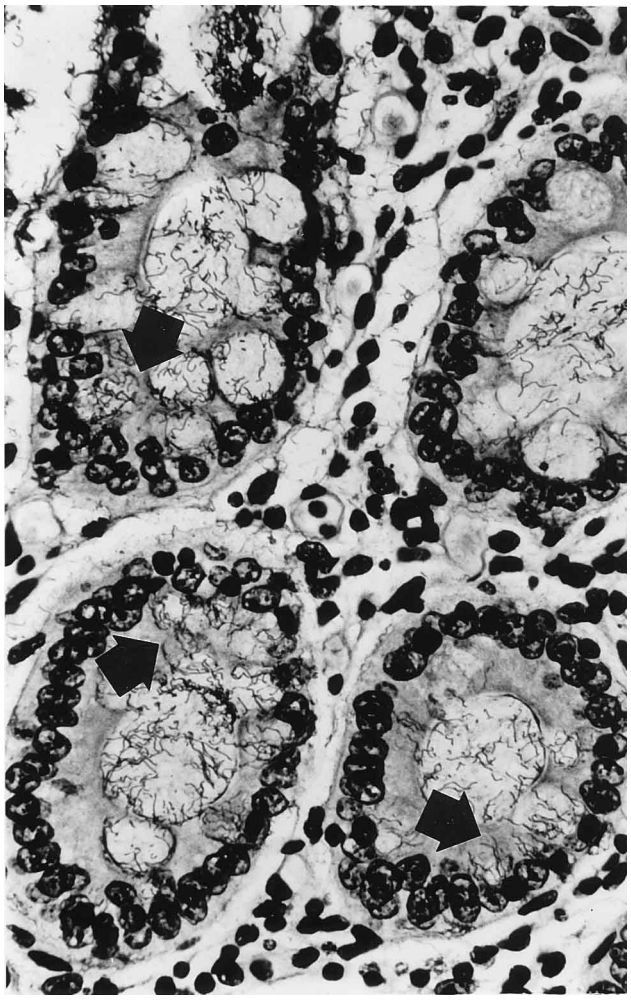

FIG. 4. Colonic glands of a pig 14 days after oral inoculation with *S. pilosicoli* P100/6/93 showing large spirochetes in or on goblet cells and epithelial cells lining the lumens (arrows). (Warthin-Starry; magnification, ×320.) This pig was diarrheic, had gross and histologic lesions of colitis, and was culture positive for *S. pilosicoli.*

tion was unknown and it is possible that this factor is of importance when considering interactions between the two infections. The presence of a normal intestinal flora does not prevent colonization of *Serpulina* spp. (7), indicating its adaptation to the pig colon. The intestinal flora may be required to produce an appropriate redox potential suitable for these anaerobic spirochetes.

While the findings of this study confirm that *S. pilosicoli* is capable of colonizing the colon of the healthy pig, we did not detect any end-on attachment to the colon epithelium but rather a loose colonization of the lumenal epithelium and the colonic glands. End-on attachment is regarded as being a pathognomonic feature of this disease (3, 23, 27). It is not clear whether the lack of obvious attachment in this study was due to strain variation, differences in intestinal flora competition in our pigs, or the stage of infection at the time of necropsy. Reexamination of field cases arising from 20 local outbreaks of *S. pilosicoli* infection, including those whence the isolates used in this study were derived, found no clear evidence of end-on attachment (24). It is possible that attachment is confined to a short period early in the course of infection and consequently not represented in this study or the vast majority of field cases submitted for necropsy. Therefore, it remains uncertain whether end-on attachment is a prerequisite for pathogenicity. Numerous studies of the pathogenesis of *S. hyodysenteriae* infection of the pig colon have demonstrated that the organism colonizes the colonic glands without specific attachment (8), indicating that attachment is not a prerequisite for development of pathology in swine dysentery.

The development of challenge models for intestinal disease due to *S. pilosicoli* is important for testing therapeutic and control measures for the naturally occurring condition. Drugs with low minimum inhibitory concentrations in vitro would still need to persist in an active form in the lower bowel to be effective. In addition, the model provides the means for more fundamental research on the pathogenesis of the disease and the role of possible contributory factors such as diet.

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