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Identification of a New Intestinal Spirochete with Pathogenicity for Chickens

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Two intestinal spirochete isolates obtained from chickens with diarrhea were examined by electron microscopy, biochemical tests, rRNA gene restriction pattern analysis, and multilocus enzyme electrophoresis. One isolate (strain 91-1207/C1) was pathogenicity tested in vivo in chickens. The chicken spirochetes were morphologically indistinguishable from *Serpulina innocens* and *Serpulina hyodysenteriae* and phenotypically similar to *S. innocens*. However, the chicken spirochetes could be distinguished from *S. innocens*, *S. hyodysenteriae*, and other swine intestinal spirochetes by rRNA gene restriction pattern analysis and multilocus enzyme electrophoresis. In pathogenicity tests in 1-day-old chicks and 14-month-old hens, chicken spirochete 91-1207/C1 produced pale-yellow, watery cecal contents and mild lymphocytic typhlitis. These findings support the conclusion that avian intestinal spirochetes can be pathogenic to commercial poultry and that the microorganisms are different from intestinal spirochetes that infect pigs.

Spirochetes have been identified in the large intestine of rodents, swine, dogs, humans, common rheas, and chickens (5, 16, 25, 26, 45). These spirochetes were either an integral part of the autochthonous flora, associated with intestinal disease, or enteropathogens. In swine, weakly beta-hemolytic *Serpulina* (*Treponema*) *innocens* (28) and nonhemolytic *Treponema succinifaciens* (4) are common nonpathogenic inhabitants of the large intestine. Other weakly beta-hemolytic intestinal spirochetes have been isolated from swine and humans with diarrhea (22, 26, 32, 34), and certain porcine isolates have been shown to be capable of inducing disease in conventional (55) and gnotobiotic swine (37). The strongly beta-hemolytic spirochete *Serpulina hyodysenteriae* produces swine dysentery, a mucohemorrhagic colitis with epithelial cell necrosis in pigs (12, 15).

Weakly beta-hemolytic spirochetes associated with diarrhea, retarded growth rate, decreased egg production, and feces-stained eggshells have been identified in chickens from Europe (5, 10, 13) and North America (53). However, the chicken spirochetes have not been characterized genotypically, and their relationship to swine or human spirochete isolates has not been established. The pathogenicity of the North American chicken-origin spirochetes is not known. In this paper, we report the phenotypic characteristics, rRNA gene restriction pattern analysis, multilocus enzyme electrophoretic (MEE) analysis, and a preliminary in vivo pathogenicity study of spirochetes isolated from chickens with diarrhea.

MATERIALS AND METHODS

Growth of spirochetes. Spirochete strains 91-1207/C1 (C1) and 91-1207/C2 (C2), isolated from the ceca of two chickens in a single commercial flock experiencing diarrhea (53), were compared with *S. hyodysenteriae* B78 (ATCC 27164) (15) and *S. innocens* B256 (ATCC 29796) for phenotypic characteristics. C1 and C2 laboratory stocks were derived through repeated restreaking from the periphery of early growth on blood agar plates, and the presence of a single spirochete isolate in each stock was confirmed by rRNA gene pattern analysis and MEE. All spirochetes, except for those used in rRNA gene restriction pattern analysis and MEE, were cultured at 37°C on 5% sheep blood agar plates (BBL, Cockeysville, Md.) in an anaerobic atmosphere produced by a GasPak Plus system envelope (BBL). After 72 h, spirochete cells were washed from the plates with physiologic saline and pelleted by centrifugation at 3,000 × *g* for 10 min at 4°C. Hemolysis patterns for plates of C1 and C2 were quantified subjectively by comparison with plates of *S. hyodysenteriae* (strongly beta-hemolytic) and *S. innocens* (weakly beta-hemolytic).

Morphologic and phenotypic characteristics. Bacterial ultrastructure was determined by examination of negatively stained whole bacterial cells (34) and plastic-embedded 90-nm uranyl acetate- and lead citrate-stained sections. Cell total lengths, diameters, height of spirals (amplitude), and length of spirals (wavelength) were determined from measurements of electron photomicrographs. Enzyme profiles and indole production were determined by a commercial kit (API ZYM; Analytab Products, Plainview, N.Y.). The enzyme profiles were coded by the method of Hunter and Wood (21).

rRNA gene restriction patterns. Spirochetes used in the rRNA gene restriction studies were *S. hyodysenteriae* B204 (ATCC 31212), *S. innocens* B256, chicken spirochetes C1 and C2, *T. succinifaciens* 6091 (ATCC 33096), nonpathogenic swine spirochete B1555a (29), and the uncharacterized porcine intestinal spirochetes 425, 31033-92, and 9724-93. Spirochetes were grown in brain heart infusion serum broth cultures under a 1% O_2 atmosphere (48), except *T. succinifaciens*, which was grown under a CO_2 atmosphere in RFG medium (47). Genomic DNA from spirochetes was isolated by the guanidium thiocyanate procedure (41). Spirochetal DNA was digested with restriction enzyme *Ssp*I, electrophoresed on a 0.7% agarose gel, blotted, hybridized with photobiotin-labelled rRNA from *S. hyodysenteriae* B204, and developed with a streptavidin-alkaline phosphatase-based system as described previously (24).

MEE. Spirochete strains C1 and C2 were examined by MEE by the methodology used previously for intestinal spirochetes from swine and humans (34, 35). Briefly, the strains were grown in prereduced anaerobic Trypticase soy broth supplemented with 2% fetal bovine serum and a 1% ethanolic cholesterol solution (31), harvested in the late log phase, and sonicated, and the lysates were subjected to electrophoresis in horizontal starch gels. The mobility of 15 constitutive enzymes was examined in relation to standards established for 188 porcine intestinal spirochetes (34). Mobility variants were interpreted as the products of different alleles at the corresponding loci. The avian spirochetes were allocated to an electrophoretic type (ET) on the basis of their allelic profile, and the genetic distances of the ET from those containing porcine isolates were calculated as the proportion of the fixed loci at which dissimilar alleles occurred. A phenogram showing the relationship of the avian isolates to those of porcine origin was constructed by the clustering fusion strategy of the unweighted pair-group method of arithmetic averages (34).

Pathogenicity for chickens. Fifteen 1-day-old specific-pathogen-free single-

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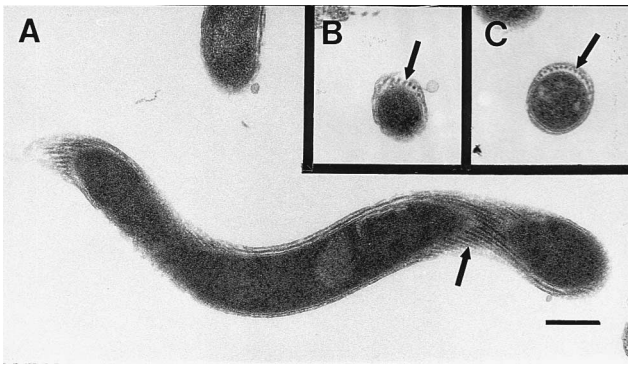

FIG. 1. Transmission electron micrograph of a C1 chicken spirochete. On longitudinal section, the spirochete (A) is helically shaped and has visible periplasmic flagella. On transverse section, an end (B) and the middle (C) of a spirochete cell have 8 and 16 periplasmic flagella, respectively. Arrows indicate periplasmic flagella. Bar, 250 nm.

comb White Leghorn chickens (chicks; Hy-vac, Gowrie, Iowa) and 10 14-month-old specific-pathogen-free single-comb White Leghorn chickens (hens; SPAFAS, Roanoke, Ill.) were divided into three treatment groups and inoculated by crop gavage (Table 2). The hens were in the beginning of a second laying cycle. Inocula for the homogenate-inoculate groups were prepared by manual homogenization (Tenbroeck tissue grinder) of ceca obtained from a chicken with intestinal spirochetosis and clinical diarrhea (53) in 1% peptone water (PW). Inocula for culture-inoculated chickens were prepared by resuspending the spirochete pellets (see "Growth of spirochetes" above) in 1% PW. Sham-inoculated chickens received 1% PW.

Chickens were weighed at days 0, 5, 10, and 21 postinoculation (p.i.), and weight gains were calculated and analyzed by one-way analysis of variance. On day 21 p.i., all birds were euthanatized with intravenous sodium pentobarbital and necropsied. Cloacal swabs (days 0, 5, and 10 p.i.) and cecal mucosa (day 21 p.i.) were cultured aerobically on brilliant green agar plates (BBL), microaerobically on Skirrow's medium agar plates (BBL), and anaerobically on antibiotic-containing modified blood agar plates (23) for isolation of *Salmonella* sp., *Campylobacter* sp., and spirochetes, respectively. The cloacal swabs from the chicks were pooled prior to culturing, but for the hens, cloacal swabs were cultured individually. All cecal samples were cultured individually. Sections of the midduodenum, midjejunum, midileum, midcecum, and midrectum were immersed in 10% neutral buffered formalin solution within 2 min of euthanasia and routinely processed to hematoxylin-and-eosin-stained 6-μm tissue sections. For the hens, additional intestinal sections were cut and stained by the Warthin-Starry silver method (30) to visualize spirochetes. Ceca from the hens were routinely processed for scanning electron microscopy (SEM) (45). The intestinal contents for each treatment group were pooled and examined by immunoelectron microscopy for rotaviruses, reoviruses, astroviruses, coronaviruses, and enteroviruses (43, 46).

RESULTS

Morphologic and phenotypic characteristics. C1 and C2 spirochetes formed tight helical coils, had blunt ends, and had a median of eight periplasmic flagella inserted subterminally in single rows at each end of the cell. These two rows of eight periplasmic flagella each overlapped in the middle 50 to 90% of the cell to produce a periplasmic flagellar ratio of 8:16:8 (Fig. 1). C1 and C2 were indistinguishable from *S. hyodysenteriae* and *S. innocens* on the basis of cell length, diameter, amplitude, and wavelength (Table 1). However, C1 and C2 differed from *S. hyodysenteriae* in several characteristics, including being weakly beta-hemolytic, having β-galactosidase, lacking α-glucosidase, and lacking indole production (Table 1). Also, the overall cytosolic enzyme profile of C1 and C2 (14.0.4.3.1) differed from that of *S. hyodysenteriae* (Table 1). C1, C2, and *S. innocens* were identical in all phenotypic characteristics (Table 1).

rRNA gene restriction patterns. Chicken spirochete C1 differed from both *S. hyodysenteriae* B204 and *S. innocens* B256 in its banding pattern (Fig. 2). In addition, the banding pattern for C1 differed from that of five other pig intestinal spirochetes, including *T. succinifaciens* 6091 (Fig. 2). C1 and C2 had identical rRNA gene restriction patterns (data not shown).

MEE. The two chicken isolates belonged to the same ET, located midway between the porcine *Serpulina* spp. isolates in groups A and B (Fig. 3) and separated from both groups at an approximate genetic distance of 0.75.

Pathogenicity for chickens. (i) Microbiology. Cloacal swabs (days 0, 5, and 10 p.i.) and cecal samples (day 21 p.i.) from all chickens were negative for *Salmonella* spp. and *Campylobacter* sp. Cloacal swabs were negative for spirochetes from all chickens on day 0 p.i. and from sham-inoculated chickens on days 5 and 10 p.i. Enteric viruses were not visualized in pooled intestinal contents by immunoelectron microscopy. Spirochetes were isolated from cloacal swabs and cecal mucosa of homogenate-inoculated chicks and hens and C1-inoculated chicks. Spirochetes were not isolated from sham-inoculated chickens (Table 2). Spirochetes were visualized by SEM in the ceca of C1-inoculated hens, but isolation attempts for spirochetes were unsuccessful (Table 2). There was no difference in weight gains between the different treatment groups (data not shown).

(ii) Gross pathology. During the 21-day experimental period, most fecal droppings were normal in color and texture for

TABLE 1. Morphologic and phenotypic characteristic of cecal spirochetes isolated from chickens (C1 and C2) and pigs (*S. hyodysenteriae* and *S. innocens*)

Characteristics	Values for bacterial species			
	C1	C2	*S. hyodysenteriae* B78	*S. innocens* B256
Periplasmic flagellar ratio	8:16:8	8:16:8	8:16:8	8:16:8
Length[a] (μm)	8.76 ± 0.78	9.28 ± 1.10	10.00 ± 2.24	9.49 ± 0.89
Diameter[a] (μm)	0.32 ± 0.02	0.30 ± 0.02	0.33 ± 0.03	0.36 ± 0.05
Amplitude[a] (μm)	0.74 ± 0.17	0.68 ± 0.28	0.58 ± 0.23	0.72 ± 0.28
Wavelength[a] (μm)	3.31 ± 0.52	4.22 ± 0.76	3.18 ± 0.42	4.02 ± 0.55
Beta-hemolysis	Weak	Weak	Strong	Weak
Indole production	−	−	+	−
Enzymes[b]				
α-Galactosidase	+	+	−	+
α-Glucosidase	−	−	+	−
API ZYM score	14.0.4.3.1	14.0.4.3.1	14.0.4.10.1	14.0.4.3.1

[a] Mean ± standard deviation for 10 to 30 spirochete cells.
[b] Alkaline phosphatase, esterase, esterase lipase, acid phosphatase, β-galactosidase, and β-glucosidase were present in all isolates. Lipase, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosamidase, α-mannosidase, phosphohydrolase, and α-fucosidase were lacking in all isolates.

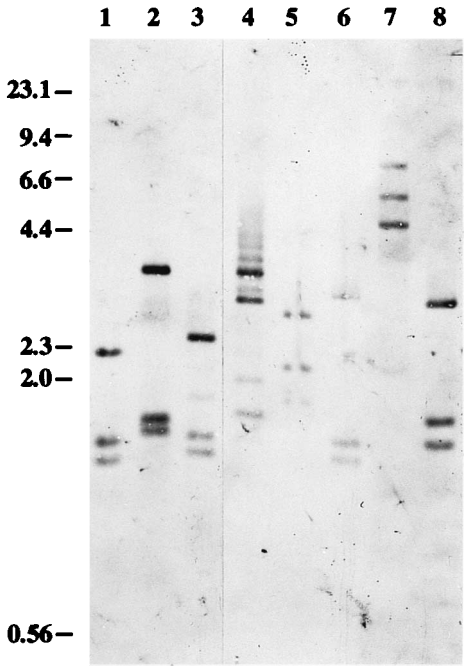

FIG. 2. *Ssp*I-digested spirochetal DNA hybridized with photobiotin-labelled *S. hyodysenteriae* B204 rRNA. Lanes: 1, *S. hyodysenteriae* B204; 2, *S. innocens* B256; 3, chicken spirochete C1; 4, swine spirochete B1555a; 5, porcine spirochete 31033-92; 6, porcine spirochete 9724-93; 7, *T. succinifaciens* 6091; 8, porcine spirochete 425. Numbers on the left are kilobase size markers.

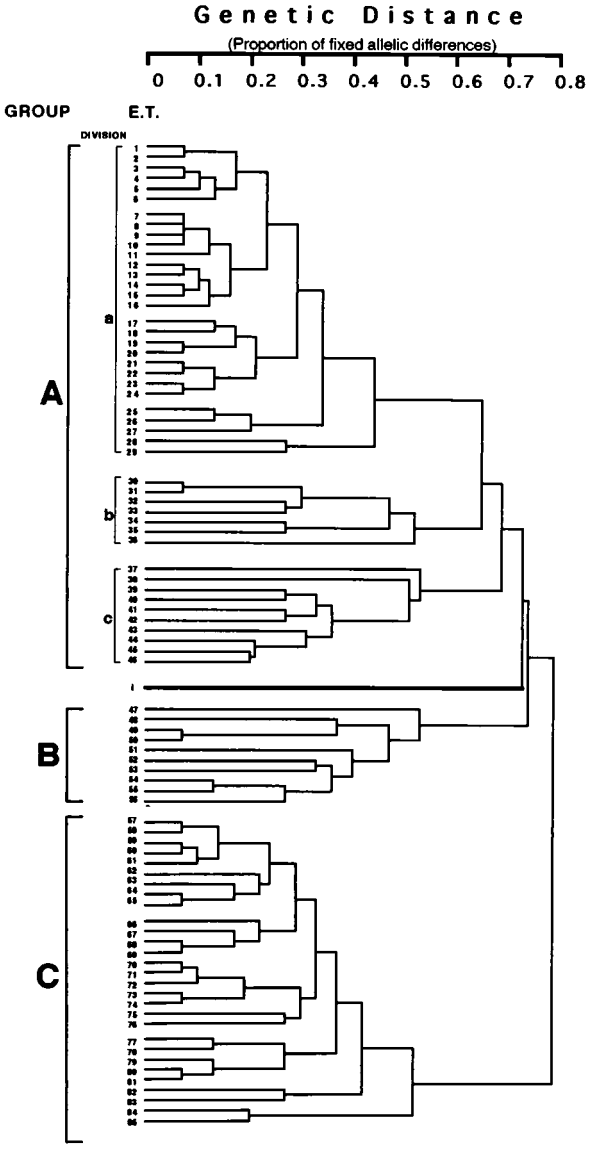

FIG. 3. Phenogram of genetic distance among ETs of 188 porcine and 2 avian intestinal spirochetes, clustered by the strategy of unweighted pair-group method of arithmetic averages. The avian spirochetes are contained in the ET outlined in bold and are distinct from groups A, B, and C. Group Aa is *S. hyodysenteriae*, and group Ac is *S. innocens*. Provisional designations for groups Ab, B, and C are "*S. intermedius,*" "*S. murdochii*," and "*Angullina coli*," respectively (33, 34).

all chicken groups. However, beginning on day 8 p.i., some fecal droppings from C1- and homogenate-inoculated chickens had occasional red flakes or a slight red discoloration. Beginning on day 5 p.i., chickens inoculated with C1 or cecal homogenate passed yellow, gold, or orange cecal droppings. The sham-inoculated chickens passed dark-green cecal droppings.

At necropsy, sham-inoculated chickens had dark-green pasty cecal contents. Ceca of C1- and homogenate-inoculated chickens were dilated and contained pale-green to yellow, fluid to frothy contents.

(iii) Histopathology. Ceca of sham-inoculated chickens had uniformly distributed villi covered by tall columnar epithelia and a minimal number of lymphocytes, plasma cells, and macrophages within the lamina propria (Fig. 4a). Spirochetes were not visualized. C1- and homogenate-inoculated chickens had mild to moderately severe lymphoplasmacytic typhlitis (Fig. 4b) and proctitis with lymphocyte and/or heterophil exocytosis, mild cecal villus epithelial cell hyperplasia, edema in the lamina propria of villar tips, and submucosal lymphocytic follicles (Table 2). In the ceca, rare to infrequent necrotic epithelial cells and mononuclear leukocytes were present within villar tips (Fig. 4c). Ceca from some homogenate-inoculated chicks had mildly dilated crypts. Histologically, cecal and rectal lesions were most severe in homogenate-inoculated chickens.

Mats of numerous spirochetes were visualized on the villar surface and within the crypts of the ceca of homogenate-inoculated hens (Fig. 4d). A few spirochetes were visualized in the ceca of C1-inoculated hens. In both C1- and homogenate-inoculated hens, spirochetes rarely invaded between or below the cecal epithelial cells. When present, the spirochetes were usually associated with necrotic epithelia of the villar tips. The small intestines of all chickens lacked histologic lesions except for mild lymphocytic ileitis in a few C1- and homogenate-inoculated chickens. Spirochetes were not visualized within the small intestines.

(iv) SEM. In the sham-inoculated hens, the mucosa of the ceca formed uniform villi covered by a smooth layer of epithelial cells. The lumenal surface of the epithelial cells was partially covered by noninvasive cocci, coccobacilli, short straight bacilli, and long curved bacilli. In C1- and homogenate-inoculated hens, the mucosal surface of the ceca exhibited mild hypertrophy of villi and widespread individual epithelial cell extrusion (Fig. 5a). The lumenal surface of the epithelial cells was covered by fibrin, debris, extruded epithelial cells, and bacteria. The bacteria were similar in numbers and morphology to those in the ceca of sham-inoculated chickens except randomly oriented spirochetes were also identified in the ceca

TABLE 2. Histologic, spirochete isolation, and SEM data for ceca of specific-pathogen-free 1-day-old chicks and 14-month-old hens inoculated by crop gavage and necropsied on day 21 p.i.

Treatment group	Inoculum[a]	*n*	No. of animals with histological findings of:			No. of animals with spirochetes in ceca by:	
			Normal	Mild lymphocytic typhlitis	Mild cecal epithelial cell hyperplasia	Culture	SEM[b]
1-day-old chicks							
Sham	1% PW	5	5	0	0	0	
C1	$10^{8.3}$ C1 spirochetes in 1% PW	5	0	3	5	5	
Homogenate	130 mg of ceca homogenized in 1% PW	5	2	3	0	5	
14-mo-old hens							
Sham	1% PW	3	3	0	0	0	0
C1	$10^{8.6}$ spirochetes in 1% PW	4	0	4	2	0	2
Homogenate	260 mg of ceca homogenized in 1% PW	3	0	3	1	2	3

[a] Hens received 1.0 ml of an inoculum (260 mg of ceca per ml of PW or $10^{8.6}$ spirochetes per ml of PW), but because of volume-induced gastric reflux, chicks received one-half the hen dose. Ceca used for the cecal homogenate were from a chicken with intestinal spirochetosis and clinical diarrhea (54).

[b] SEM was not performed on the ceca of 1-day-old chicks.

of C1- and homogenate-inoculated chickens (Fig. 5b). Spirochetes were numerous in homogenate-inoculated hens and sparse in C1-inoculated hens, but in both groups, the spirochetes rarely penetrated between epithelial cells. Small spiral bacteria with single terminal flagellae were also present in the ceca of homogenate-inoculated chickens.

DISCUSSION

This study demonstrated colonization of chicken ceca by a weakly beta-hemolytic spirochete originally isolated from White Leghorn chickens with diarrhea and typhlitis (53). The chicken-origin C1 spirochete colonized the ceca and formed mats of randomly oriented spirochetes associated with the lumenal surface of the epithelium. Similar random surface colonization has been documented for *S. hyodysenteriae* and *S. innocens* in swine colons (27). In the current study, the chicken spirochete was morphologically indistinguishable from *S. innocens* and *S. hyodysenteriae* and phenotypically similar to *S. innocens*. However, the C1 spirochete was phenotypically distinct from *S. hyodysenteriae* by being weakly beta-hemolytic, having α-galactosidase, lacking α-glucosidase, and not producing indole. *S. hyodysenteriae* was strongly beta-hemolytic, lacked α-galactosidase, had α-glucosidase, and produced indole.

The C1 chicken-origin spirochete was distinct from *S. innocens*, *S. hyodysenteriae*, and other unclassified swine intestinal spirochetes as demonstrated by rRNA gene restriction pattern analysis. It is not clear whether the differences in rRNA gene restriction patterns are significant enough to place the chicken spirochetes in a separate group apart from the porcine spirochetes, although this possibility is supported by the results of MEE. rRNA gene restriction pattern analysis has been proposed as a method to differentiate bacterial species within the same genus (38, 56) as well as for evaluating differences within a species (2, 11, 40). For spirochetes, rRNA gene restriction pattern analysis has allowed reliable identification and differentiation of strains of *S. hyodysenteriae*, identification of unknown spirochete isolates as strains of *S. hyodysenteriae*, and differentiation of unknown spirochetes from *S. hyodysenteriae*, *S. innocens*, and other porcine intestinal spirochetes (24).

Recently, MEE has been used to separate intestinal spirochetes of swine and humans into genotypic groups, namely, Aa, Ab, Ac, B, and C (34, 35). Group A and B spirochetes were morphologically similar and, during colonization of the large intestine, were oriented randomly within the mucous gel adjacent to the lumenal surface of the epithelium. In comparison with spirochetes of groups A and B, group C spirochetes had fewer periplasmic flagella, smaller mean cell diameters, and colonized the lumenal microvillus zone of the colonic epithelium by end-on-end attachment (34). MEE groups Aa and Ac correspond to *S. hyodysenteriae* and *S. innocens*, respectively. MEE groups Ab, B, and C have not been classified taxonomically but have been designated provisionally as "*S. intermedius*," "*S. murdochii*," and "*Angullina coli*", respectively (33), and are currently being examined for characteristics traditionally used in spirochete classification.

In the current study, the C1 and C2 American chicken spirochetes belonged to the same MEE ET and were distinct from spirochetes of porcine and human origin in groups A, B, and C (14, 34, 35) as well as from another series of weakly beta-hemolytic porcine intestinal spirochetes that have been examined recently (14, 37). European and Australian chicken intestinal spirochetes were similar to the two American chicken isolates in hemolysis pattern, numbers of periplasmic flagella, diameter, length, and amplitude (5), but the European and Australian chicken spirochetes were different from the American spirochetes because they had α-glucosidase, lacked α-galactosidase, and typed in the MEE group Ab ("*S. intermedius*") (36). On the basis of MEE, the American chicken spirochetes were most closely related to *S. innocens* and group B spirochetes and may represent a new species in the genus *Serpulina*. DNA-DNA homology and 16S rRNA sequence studies must ultimately be accomplished to determine the phylogenetic relatedness between the American chicken spirochetes and other intestinal spirochetes. Because C1 and C2 were isolated from chickens on the same poultry farm and had identical phenotypic and genotypic characteristics, C1 and C2 were interpreted as being the same spirochete strain.

In the preliminary in vivo study, the weakly beta-hemolytic C1 chicken spirochete was mildly pathogenic for chickens. In field cases in chickens from Europe and North America, cecal spirochete infection was associated with significant disease, retarded growth rates, delayed onset of egg production, diarrhea, and the production of feces-stained eggshells (5, 13, 53). In our study, spirochete-infected chickens had discolored intestinal and cecal droppings, watery to foamy pale cecal contents, and mild typhlitis and proctitis. Such cecal and rectal

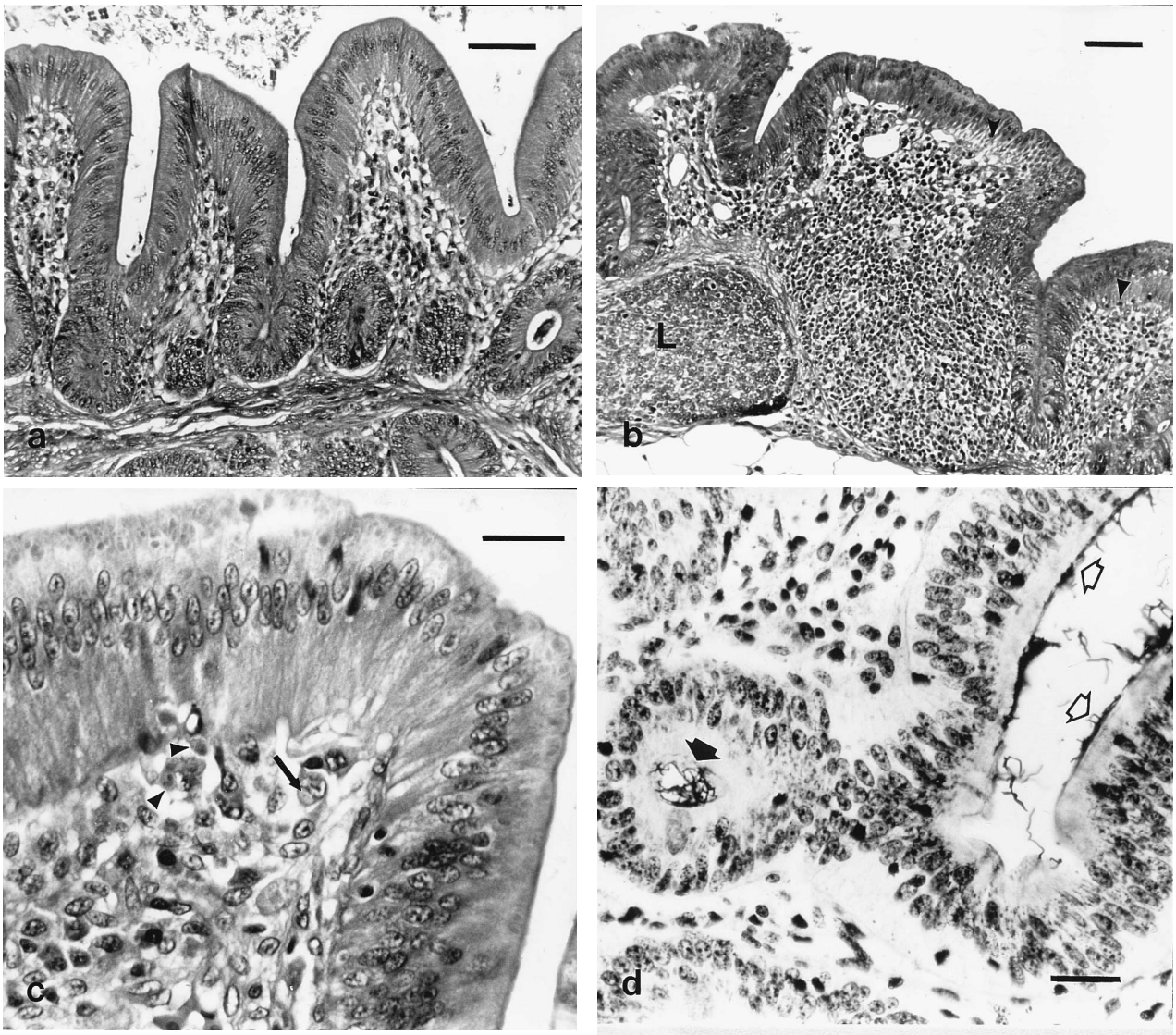

FIG. 4. Light micrographs of mid-cecum sections of sham- (a) and homogenate- (b, c, and d) inoculated hens 21 days p.i. (a) Normal mucosa and submucosa; (b) mucosa and submucosa showing mild lymphocytic typhlitis with mild epithelial hyperplasia, lymphocyte exocytosis (arrowheads), and a submucosal lymphocytic follicle (L); (c) necrotic cells (arrowheads) and phagocytized debris (arrow) within villar tips; (d) numerous spirochetes in a crypt (filled arrow) and on the surface epithelium (open arrows). Stains, hematoxylin and eosin (a, b, and c) and Warthin-Starry silver (d). Bars, 50 (a and b) and 20 (c and d) μm.

lesions consisted most frequently of lymphocyte and plasma cell infiltrates in the lamina propria, crypt distension with sloughed epithelial cells and spirochetes, hyperplasia of epithelial cells, and exocytosis with mixed inflammatory cells. Similar gross and histologic changes have been reported in Europe in chickens inoculated with weakly beta-hemolytic chicken-origin cecal spirochetes and in both Europe and North America for naturally infected chickens (5, 8, 13, 53). However, in European studies, the chicken-origin spirochetes were more pathogenic as evident by reduced weight gain, more frequently identified epithelial cell necrosis, and more constant appearance of subepithelial spirochetes within the cecal lamina propria (6–8, 54). The reason for the greater severity of disease and lesions produced by European chicken spirochete isolates is unknown but may have resulted from utilizing the more susceptible broiler chicken model or a crude cecal inoculum containing spirochetes plus copathogenic organisms. In addition, the MEE analysis and some phenotypic characteristics suggest that the European chicken spirochetes are a different species from C1 and thus potentially express greater pathogenicity (6, 9, 36). Other intestinal spirochetes have greater pathogenicity than C1. For example, strongly beta-hemolytic *S. hyodysenteriae* produces severe mucosal necrosis, intense inflammation in the lamina propria, and invasion of spirochetes in the colon and ceca of experimentally inoculated pigs and chickens, respectively (1, 12, 50–52, 58).

The lower recovery rate of spirochetes from inoculated hens than chicks in the current study may be the result of competitive exclusion by normal cecal microflora of the hens. At the time of hatching, the gastrointestinal tract of the chick is essentially devoid of bacteria and, as the bird matures, a normal intestinal microflora develops and thrives (44). The predominant cecal flora in the chicken is aerobic enterococci and *Escherichia coli* at day 1 posthatching, *E. coli* at day 3 posthatching, and anaerobes at days 14 and 28 posthatching (20). This normal intestinal flora can exert a competitive and exclusive action

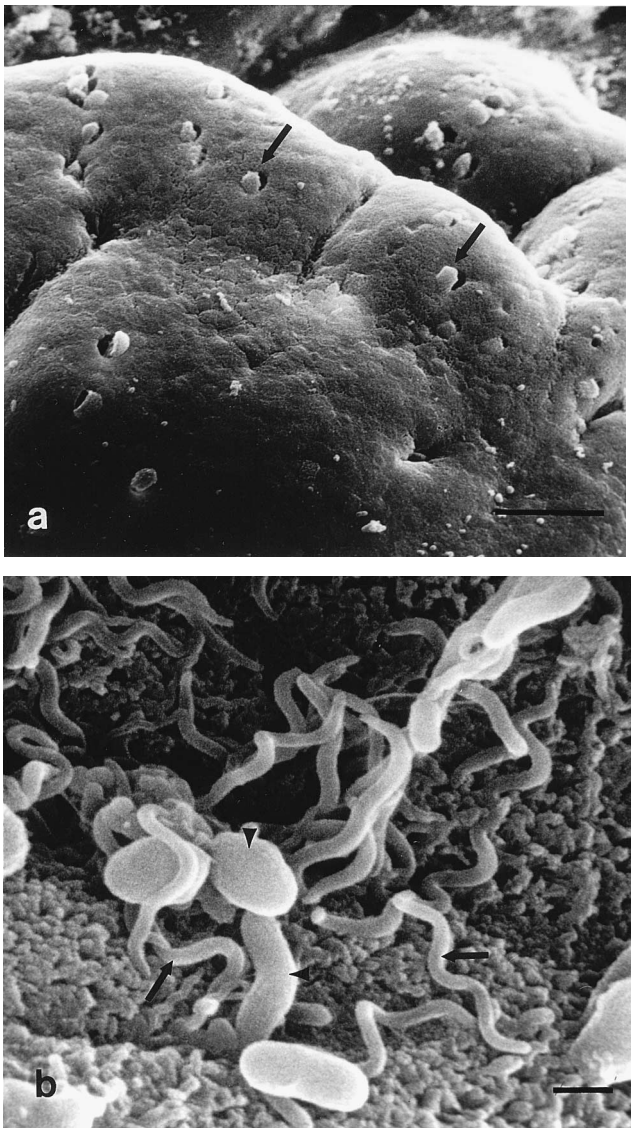

FIG. 5. SEMs of midcecal samples from a homogenate-inoculated chicken, 21 days p.i. (a) Hyperplastic villi with lumenal extrusion of surface epithelial cells (arrows); (b) surface covered by a mat of randomly oriented, noninvasive spirochetes (arrows) and a few other miscellaneous bacteria (arrowheads). Bars, 20 (a) and 1 (b) μm.

which can protect the enteric tract from colonization and multiplication of pathogenic bacteria (44). For example, inoculation of 1- to 8-day-old chicks with either undefined normal cecal bacteria, *E. coli*, or *Lactobacillus* sp. reduced or prevented colonization of the ceca by pathogenic *Salmonella* sp. (3, 39, 49, 57). In the current study, such competitive exclusion could have accounted for the lower spirochete infection rate and less frequent visualization of spirochetes in the ceca of hens than of chicks. However, natural cases of cecal spirochetosis in laying hens have been associated with large numbers of lumenal spirochetes (5, 13, 53) and may indicate the necessity of cofactors to produce pathologic effect and clinical disease. In one field study, the spirochete infection was associated with recovery of hens from a forced molt used to induce a second laying cycle (53). In studies with another pathogenic bacterium, *Salmonella enteritidis*, forced molt resulted in a 10^2 to 10^3 lower mean infection dose, a 100- to 1,000-fold increase in organisms within intestinal secretions, and more severe histologic lesions in the ceca than were produced in nonmolting hens (17–19, 42). Forced molting or other stressors may have produced hens more susceptible to spirochete infections and resulted in a more severe disease than that found in nonstressed hens.

In summary, the current study demonstrated that a chicken-origin cecal spirochete was mildly pathogenic for 1-day-old chicks and 14-month-old hens. This spirochete, although similar to *S. hyodysenteriae* and *S. innocens* in many morphologic and phenotypic characteristics, was distinctly different on the basis of rRNA gene restriction pattern analysis and MEE. Definitive classification of the organism awaits more detailed molecular characterization.

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