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## Ileocolitis Associated with *Anaerobiospirillum* in Cats†

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**Ileocolitis associated with spiral bacteria identified as an *Anaerobiospirillum* sp. was found in six cats. Two cats had acute onset of gastrointestinal signs characterized by vomiting and diarrhea in one cat and vomiting in another cat, one cat had chronic diarrhea that was refractory to medical therapy; one cat had acute onset of anorexia and lethargy, and two cats had clinical signs that were not related to the gastrointestinal tract. The presence of an *Anaerobiospirillum* sp. was demonstrated on the basis of ultrastructural morphology of spiral bacteria associated with intestinal lesions and PCR amplification of a genus-specific 16S rRNA gene from affected tissues from each cat. The colons of three clinically healthy cats without lesions and one cat with mild colitis not associated with spiral bacteria were negative for *Anaerobiospirillum* spp. in the same assay. Comparative nucleotide sequence analysis of cloned PCR products from three affected cats further suggested that the spiral bacteria were closely related to *Anaerobiospirillum succiniciproductens*.**

*Anaerobiospirillum* is a genus of small, spiral gram-negative motile bacteria that have been isolated from the throat and feces of healthy dogs and cats (2, 10, 13), and diarrheal feces (12, 16) and blood (11, 18, 23, 27) of humans. The genus *Anaerobiospirillum* comprises two species, *A. succiniciproductens* and *A. thomasi*, but only *A. succiniciproductens* has been associated with septicemia in humans (10). Bacteremia in humans appears to be found almost exclusively in subjects with underlying disease, and immunosuppression is a risk factor (15). There is only one report of bloody diarrhea in a puppy from which an *Anaerobiospirillum* species was isolated in combination with other species of spiral bacteria (16).

In this report, we describe six cats that had either ileitis, ileocolitis, or colitis associated with the presence of spiral bacteria. Three cats were presented with gastrointestinal signs characterized by vomiting or diarrhea, one cat was presented with an acute onset of anorexia and lethargy, and two cats had no clinical or systemic signs related to the gastrointestinal tract. In all cases, spiral bacteria identified as an *Anaerobiospirillum* species based on morphological and gene-specific PCR assays were associated with intestinal lesions. Comparative sequence analysis of partial 16S rRNA gene sequences obtained from intestinal tissues from three of the affected cats revealed *Anaerobiospirillum* spp. related to but different from the previously cultured canine reference *A. succiniciproductens* strain.

This is the first pathology description of *Anaerobiospirillum* ileocolitis in any host.

### CASE REPORTS

**Case 1.** A 14-year-old male neutered domestic shorthair cat was presented to the emergency service at the Veterinary Medical Teaching Hospital (VMTH) for acute onset of vomiting, diarrhea, and lethargy (Table 1). Physical examination revealed marked hypothermia, bradycardia, and hypotension. Painful, thickened intestinal loops were palpated, and hematochezia was noticed during the examination. Shock treatment was unsuccessful. Given a guarded prognosis for the cat, the owner elected euthanasia.

**Case 2.** A 21-year-old male neutered domestic shorthair cat was presented to the VMTH emergency service for acute onset of pelvic limb paralysis. Physical examination revealed dyspnea, cyanosis, irregular heart rate, and the absence of deep pain, withdrawal reflexes, and femoral pulses in both pelvic limbs. Thoracic radiographs revealed a diffuse pulmonary interstitial to alveolar pattern consistent with pulmonary edema and an enlarged cardiac silhouette. The cat was euthanized due to its rapid deterioration and poor prognosis.

**Case 3.** A stray, female domestic longhair kitten was presented to the VMTH emergency service with a 1-day history of anorexia and lethargy. On physical examination, the cat was obtunded, hypothermic, and dehydrated. Blood examination showed hypoglycemia (46 mg/dl; normal, 73 to 134 mg/dl) and anemia (packed cell volume, 19%; normal, 24 to 45%). The kitten was administered intravenous fluids with glucose; however, because of its comatose condition, the owner elected euthanasia.

**Case 4.** A 2-month-old, male domestic longhair cat was presented to the VMTH surgery service for surgical repair of a peritoneal-pericardial hernia. After an apparently uneventful recovery from the anesthesia, the cat went into respiratory

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TABLE 1. Necropsy results for cats with ileocolitis associated with *Anaerobiospirillum* species

Cat no.	Intestinal lesions	<i>Anaerobiospirillum</i> location	Mean <i>Anaerobiospirillum</i> diam, $\mu\text{m}$ (range) <sup>a</sup>	Other pathology
1	Acute necrotizing ileitis and lymphoid depletion	Crypts, ileum	0.55 (0.55–0.60)	Cardiomyopathy
2	Subacute necrotizing colitis and marked lymphoid necrosis	Crypts, colon	ND	Cardiomyopathy
3	Acute necrotizing colitis	Crypts, colon	0.58 (0.55–0.60)	Tonsils: intranuclear eosinophilic inclusions consistent with herpesvirus infection
4	Subacute, multifocal ulcerative colitis and ileitis and focal lymphoid necrosis	Crypts, ileum and colon	0.49 (0.4–0.57)	Pneumothorax and pneumopericardium
5	Subacute, diffuse pleocellular colitis and ileitis	Crypts, ileum and colon	0.45 (0.42–0.49)	Disseminated intravascular coagulation
6	Subacute, multifocal ulcerative colitis	Crypts, colon	ND	

<sup>a</sup> Determined by transmission electron microscopic examination. ND, not determined.

arrest a few hours postsurgery. The cat was placed on a respirator, and shock therapy was initiated. However, because of the poor response to therapy, the owner requested that the cat be euthanized.

**Case 5.** A 5-year-old male neutered domestic shorthair cat was presented to the VMTH emergency service for acute onset of lethargy and vomiting. The cat was part of a group of 40 indoor cats and had had a recent episode of vomiting. A blood examination by the referring veterinarian revealed neutrophilia (29,281/ $\mu\text{l}$ ; normal, 2,500 to 8,500/ $\mu\text{l}$ ), azotemia (138 mg/dl; normal, 14 to 36 mg/dl), increased creatinine (8.0 mg/dl; normal, 0.6 to 2.4 mg/dl), hyperphosphatemia (10.1 mg/dl; normal, 2.4 to 8.2 mg/dl), hyponatremia (136 meq/liter; normal, 145 to 158 meq/liter), and hypochloremia (83 meq/liter; normal, 104 to 128 meq/liter). Urine analysis was unremarkable. The cat died during physical examination.

**Case 6.** A 6-month-old kitten was evaluated for diarrhea lasting 24 h and characterized by loose, mucoid stools. A fecal flotation and direct wet preparation revealed hookworm ova and *Giardia duodenalis* trophozoites, respectively; however, appropriate anthelmintic therapy (pyrantel pamoate) and antiprotozoal therapy (metronidazole) failed to resolve the diarrheal illness. In spite of administration of sulfasalazine, gentamicin, and bismuth subsalicylate, the diarrhea persisted for an additional 12 months after the initial presentation, and the owner elected euthanasia. The kitten was negative for feline leukemia virus by commercially available enzyme-linked immunosorbent assay of the cat's serum (CITE FeLV; IDEXX Inc., Westbrook, Maine).

#### MATERIALS AND METHODS

**Light and electron microscopy.** A complete necropsy examination of each cat was done within the first 12 h after death either at the Department of Pathology, Microbiology and Immunology of the University of California-Davis (cats 1 to 5) or the Veterinary Diagnostic Center at the University of Nebraska-Lincoln (cat 6). Tissues were collected from all organs (except eyes and spinal cord) and immediately fixed in 10% buffered formalin, processed by standard methods for

paraffin-embedding, sectioned at 4.0  $\mu\text{m}$ , and stained with hematoxylin and eosin. Sections of ileum and colon were also stained with Steiner silver stain, Brown and Brenn Gram stain, and Giemsa stain. The ultrastructural morphology and distribution of bacteria in cats 1, 3, 4, and 5 were determined by transmission electron microscopic (TEM) examination of selected specimens. Blocks of ileum and colon, initially placed in 10% neutral buffered formalin, were transferred to half-strength modified Karnovsky's solution (M. J. Karnovsky, *J. Cell Biol.* 27: 270, 1965, abstr. 137A) before two washes with 0.2 M sodium cacodylate and postfixation in 2% osmium tetroxide reduced with 2.5% potassium ferrocyanide (20). Following fixation, tissues were washed in 0.2 M sodium cacodylate and dehydrated through a graded ethanol series before infiltration in Spurr's epoxy resin. Thin sections were cut and stained with 6% methanolic uranyl acetate and counterstained with lead citrate.

**DNA extraction for PCR analysis.** Total DNA was extracted from formalin-fixed and paraffin-embedded tissue blocks as previously described (3). Briefly, four 25- $\mu\text{m}$  sections of tissue were dewaxed with xylene, washed with ethanol, and dried. Lysis buffer, consisting of 50 mM Tris-HCl (pH 8.5), 1 mM EDTA, 0.5% *N*-lauroyl sarcosine, and 200  $\mu\text{g}$  of proteinase K/ml, was added, and the samples were incubated at 60°C for 48 to 60 h with an additional 200  $\mu\text{g}$  of proteinase K/ml added every 10 h for a total of four to five additions. The lysis buffer was inactivated by heating, and insoluble debris were removed by centrifugation at 16,000  $\times g$  for 60 s. The supernatant was extracted with phenol and chloroform, the DNA was precipitated with ethanol, and the pellet was dissolved in TE buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA).

***Anaerobiospirillum* genus 16S rRNA gene PCR assay.** Primers for amplification of *Anaerobiospirillum* genus 16S rRNA gene-specific sequences were designed for this study on the basis of *A. succiniciproducens* ATCC 29305<sup>T</sup> (U96412) and *A. thomasi* DSM 11806<sup>T</sup> (AJ420985) sequences. An 18-base forward primer, designated Ana16SF1, 5'-CTAATACCGCATACTCCC-3' (positions 168 to 185 in the *Escherichia coli* nomenclature [J01859]), and an 18-base reverse primer, designated Ana16SR1, 5'-TTTACGCCAGTTATTCC-3' (positions 556 to 573 in the *E. coli* nomenclature [J01859]), were designed to amplify a variable 371-nucleotide region of the *A. succiniciproducens* 16S rRNA gene.

The primers were used for PCR amplification of DNA obtained from each specimen with a thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.) in a total volume of 50  $\mu\text{l}$  containing 1.5 mM MgCl<sub>2</sub>, 1 $\times$  PCR buffer, 0.2 mM each dATP, dTTP, dGTP, and dCTP, 1.0  $\mu\text{M}$  primer, and 1.5 U of *Taq* DNA polymerase (USB Corp., Cleveland, Ohio) in filtered, autoclaved water. Initial denaturation was for 5 min at 94°C, followed by 30 cycles of 60 s at 94°C, 60 s at 55°C, and 60 s at 72°C. The ethidium bromide-stained PCR products were visualized under UV light after electrophoresis in a 1.5% agarose gel.

In preliminary studies, the specificity of the PCR assay was evaluated with pure cultures of reference *A. succiniciproducens* strain ATCC 29305<sup>T</sup> (2, 23), *A. thomasi* strain ATCC 700432<sup>T</sup> (10), and other enteric spiral organisms, including

TABLE 2. *Anaerobiospirillum* 16S rRNA gene sequences investigated in this study

Strain or tissue	Source <sup>a</sup> (GenBank no.)	Length (bp)	Reference(s)
<b>Reference strains</b>			
<i>Anaerobiospirillum succiniciproducens</i>	ATCC 29305 <sup>T</sup> (U96412)	335	2, 23
<i>Anaerobiospirillum succiniciproducens</i>	ATCC 29305 <sup>T</sup> , clone 1	335	This report
<i>Anaerobiospirillum thomasi</i>	DSM 11806 <sup>T</sup> (AJ420985)	341	8, 10
<i>Anaerobiospirillum thomasi</i>	ATCC 700432 <sup>T</sup> , clone 1	341	This report
<i>Succinivibrio dextrinosolvens</i>	DSM 3072 (Y17600)	333	8
<i>Succinimonas amylolytica</i>	DSM 2873 <sup>T</sup> (Y17599)	363	8
<i>Ruminobacter amylophilus</i>	ATCC 29744 <sup>T</sup> (Y15992)	364	14
<b>Intestinal tissues</b>			
Cat 1	Clone 1 (AY548466)	335	This report
	Clone 2 (AY548467)	335	This report
	Clone 3 (AY548468)	335	This report
Cat 2	Clone 1 (AY548469)	337	This report
	Clone 2 (AY548470)	335	This report
	Clone 3 (AY548471)	336	This report
Cat 6	Clone 1 (AY548472)	335	This report

<sup>a</sup> GenBank accession numbers of reference strains and cloned nucleotide sequences obtained by PCR amplification of DNA extracted from formalin-fixed and paraffin-embedded intestinal tissues.

*Brachyspira pilosicoli* strain P43/6/78<sup>T</sup> ATCC 51139 (24), *Helicobacter hepaticus* strain 3B1/Hh-1 ATCC 51449 (6), *Arcobacter skirrowii* strain LMG 6621 ATCC 51132 (25), *Campylobacter fetus* subsp. *fetus* strain A6629 ATCC 33246 (1), and *Escherichia coli* TOP10 (Invitrogen Corp., Carlsbad, Calif.). In each assay, pure cultures of reference *A. succiniciproducens* strain ATCC 29305<sup>T</sup> and *A. thomasi* strain ATCC 700432<sup>T</sup> and sterile water were included as positive and negative controls, respectively. Additionally, DNA extracted from formalin-fixed and paraffin-embedded intestinal tissues from a cat with mild colitis not associated with spiral bacteria (UNL14256-97) and cats with normal colons (UCD02N200 and UCD02N256; UNL14055-03) were included as negative controls.

**Cloning and sequencing of *Anaerobiospirillum* 16S rRNA gene-specific PCR products.** Amplified PCR products were excised from agarose gels and prepared for cloning (Amicon Bioseparations Ultrafree-DA ultrafiltration; Millipore Corp., Bedford, Mass.) into the plasmid vector pCR4-TOPO and transformed into chemically competent *E. coli* TOP10 (Invitrogen Corp.). The nucleotide sequences of both strands from one to three clones from each reaction were determined at the Iowa State University DNA Sequencing and Synthesis Facility (Ames, Iowa) by an automated dideoxy sequencing method (21). Reactions were carried out with the Applied Biosystems Prism BigDye terminator cycle sequencing kit with AmpliTaq DNA polymerase, fluorescent sequencing, and electrophoresis on an Applied Biosystems Prism 377 DNA sequencer.

**Data analysis and phylogenetic tree construction.** The 335- to 364-nucleotide sequences obtained from each clone and the corresponding *Anaerobiospirillum* 16S rRNA gene sequences deposited in GenBank (see Table 2) were aligned and edited manually with SeqWeb version 2.0.2 (Accelrys, Inc., Apache Software Foundation, <http://www.apache.org>) and the Wisconsin Package version 9.0 computer program (Genetics Computer Group, Madison, Wis.). Corresponding closely related 16S rRNA gene sequences from non-*Anaerobiospirillum* species were included in the analysis. These included *Succinivibrio dextrinosolvens* DSM 3072 (Y17600) (8), *Succinimonas amylolytica* DSM 2873<sup>T</sup> (Y17599) (8), and *Ruminobacter amylophilus* ATCC 29744<sup>T</sup> (Y15992) (14). Similarity values for all sequence pairs were computed with the AlignX module in Vector NTI Advance. For phylogeny estimates, partial 16S rRNA gene sequences were aligned with Clustal W (version 1.7) (7). The alignment output from Clustal W then was used for construction of the phylogenetic tree with the DNAML program within the PHYLIP 3.5c computer package (5), a program that implements the maximum-likelihood method for DNA sequences (4). The tree file from DNAML was read with TreeView, a program for displaying and printing phylogenies (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). Bootstrap values were estimated based on 100 bootstrapping data sets, generated by the SEQBOOT program of PHYLIP.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the seven cloned 16S rRNA gene PCR-amplified products obtained with DNA extracted from the intestines of cats 1, 2, and 6 have been deposited in GenBank under the accession numbers presented in Table 2.

## RESULTS

**Pathology.** The pathological findings for each cat are presented in Table 1. Significant changes were limited to the ileum in cat 1, the colon in cats 2, 3, and 6, and both in cats 4 and 5. The most significant and consistent histological change was present in the colon and consisted of marked multifocal to diffuse dilations of the crypt lumina that were filled with large numbers of spiral bacteria, often accompanied by necrotic epithelial cells and degenerate leukocytes or associated with crypt abscesses consisting of necrosis of the crypt epithelium and accumulation of polymorphonuclear neutrophils. While the bacteria were difficult to see in hematoxylin- and eosin-stained sections, special stains, such as Giemsa and Steiner, displayed large clusters of bacteria with a spiral morphology (Fig. 1). The

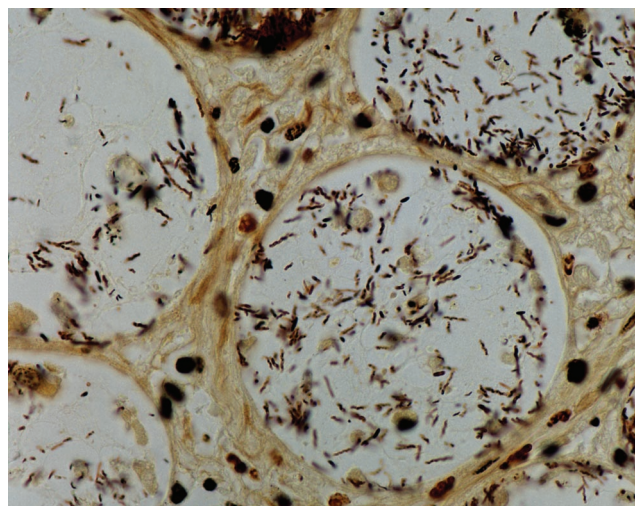


FIG. 1. Light photomicrograph of colon obtained from cat 5, showing spiral bacteria inside the lumen of a dilated crypt (Steiner stain). Magnification,  $\times 120$ .

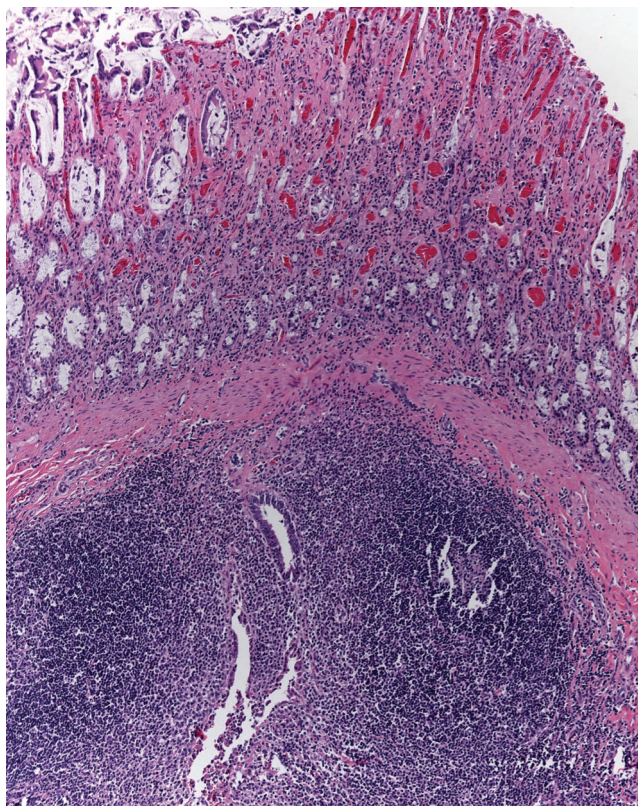


FIG. 2. Light photomicrograph of colon obtained from cat 2 with subacute colitis characterized by scarring above submucosal lymphoid aggregate and necrosis of germinal center (hematoxylin and eosin stain). Magnification,  $\times 40$ .

bacteria were also gram negative. Spiral bacteria were occasionally seen within goblet cells or free within the subepithelial connective tissue. Changes in the surface epithelium ranged from focal sloughing to multifocal erosions, particularly over submucosal lymphoid aggregates (Fig. 2) to diffuse epithelial necrosis and mucosal ulceration. Inflammatory cells were usually relatively sparse and consisted mainly of lymphocytes, with fewer histiocytes and polymorphonuclear neutrophils.

Lesions in the ileum were similar to those present in the colon and ranged from minimal epithelial damage with predominantly crypt abscesses associated with numerous spiral bacteria to diffuse epithelial necrosis. When epithelial necrosis was prominent in the ileum and colon, hemorrhage and fibrosis (Fig. 2) were also present in the subepithelial mucosa. In all cats, marked hyperplasia of the submucosal lymphoid tissue of the ileum and colon was present. In areas where necrosis of the superficial epithelium was found, necrosis of the germinal centers was also present (Fig. 2), and in cat 1, spiral bacteria were observed in the necrotic lymphoid follicles. Lesions in the other organs of these cats are summarized in Table 1. Cat 5 had diffuse intravascular coagulation and a leukocytosis which is indicative of sepsis. Ultrastructural examination revealed large numbers of spiral bacteria within the lumen of the intestinal crypts. The bacteria had a mean diameter of between 0.45 and 0.58  $\mu\text{m}$  (Table 1) with lophotrichous flagellation (Fig. 3). The pole with the flagella was flattened, with flagellar disks

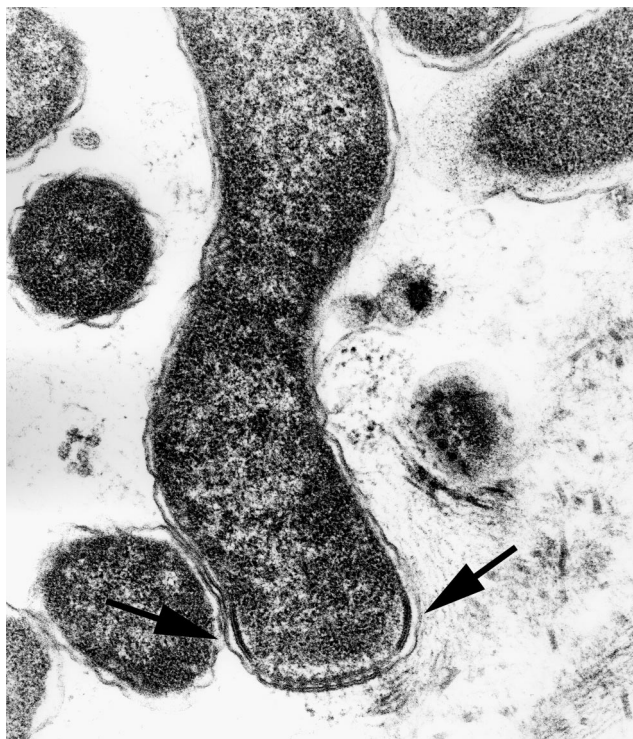


FIG. 3. Transmission electron photomicrograph of ileum obtained from cat 1 with spiral bacteria in the crypts. The lophotrichous flagellation, flattened flagellar disks embedded in less-dense cytoplasm, and electron-dense structures underneath the sheath (arrows) located peripherally at the flagellar pole are characteristic of *Anaerobiospirillum* species. Magnification,  $\times 57,375$ .

embedded in less-dense cytoplasm (Fig. 3). A linear structure composed of a dense inner region and less contrasted structure underneath the sheath was always located peripherally at the flagellar pole.

***Anaerobiospirillum* genus 16S rRNA gene PCR assay.** Total DNA extracted from the colons of each cat yielded specific 371-bp products by PCR amplification. The products from three cats were selected for cloning and comparative nucleic acid sequence analysis along with a clone from the *A. succiniciproducens* and *A. thomasii* reference strains. Attempts at amplification of either non-*Anaerobiospirillum* reference bacteria or DNA obtained from the colons of negative control cats yielded no products with the *Anaerobiospirillum* genus-specific 16S rRNA gene PCR assay.

**Comparative nucleic acid sequence analysis.** Phylogenetic analysis of partial *Anaerobiospirillum* genus 16S rRNA gene-specific sequences obtained from affected tissues of cats 1, 2, and 6 revealed that bacteria closely related to *A. succiniciproducens* were present in each cat. The results of pairwise analyses of partial *Anaerobiospirillum* 16S rRNA gene sequences are presented in Table 3 and the phylogenetic tree is presented in Fig. 4. Clones 1, 2, and 3 from cat 1, clone 2 from cat 2, and clone 1 from cat 6 had sequences similar to each other and distinct from clones 1 and 3 from cat 2 and the reference *A. succiniciproducens*. The genetic distance between this group and *A. thomasii* was 0.91 to 0.93. As expected, cloned *A. succiniciproducens* strain ATCC 29305<sup>T</sup> and *A. thomasii* strain

TABLE 3. Similarity values for pairwise partial 16S rRNA gene sequences of reference and cloned PCR products obtained from the intestines of cats with ileocolitis associated with spiral bacteria and comparison with corresponding 16S rRNA gene sequences from related bacteria

Sequence no.	Identification	Similarity to sequence:																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14			
1	<i>A. succiniciproducens</i> ATCC 29305 <sup>T</sup> [U96412] dog	1.00																
2	<i>A. succiniciproducens</i> ATCC 29305 <sup>T</sup> , clone 1 dog	1.00	1.00															
3	Cat 2, clone 1	0.99	0.99	1.00														
4	Cat 2, clone 3	0.99	0.99	1.00	1.00													
5	<i>A. thomasi</i> DSM 11806 <sup>T</sup> [AJ420985], human	0.92	0.92	0.91	0.92	1.00												
6	<i>A. thomasi</i> ATCC 700432 <sup>T</sup> clone 1, human	0.92	0.92	0.92	0.92	0.99	1.00											
7	<i>S. dextrinosolvens</i> DSM 3072 [Y17600], cow	0.94	0.94	0.93	0.93	0.93	0.93	1.00										
8	<i>S. amylolytica</i> DSM 2873 <sup>T</sup> [Y17599], cow	0.85	0.85	0.84	0.84	0.84	0.85	0.84	1.00									
9	<i>R. amylophilus</i> ATCC 29744 <sup>T</sup> [Y15992], cow	0.85	0.85	0.84	0.84	0.84	0.85	0.82	0.86	1.00								
10	Cat 1, clone 2	0.98	0.98	0.97	0.98	0.93	0.93	0.94	0.85	0.84	1.00							
11	Cat 1, clone 3	0.98	0.98	0.97	0.97	0.93	0.93	0.94	0.85	0.84	1.00	1.00						
12	Cat 6, clone 1	0.97	0.97	0.96	0.97	0.92	0.93	0.94	0.84	0.83	0.99	0.99	1.00					
13	Cat 1, clone 1	0.98	0.98	0.97	0.97	0.93	0.93	0.94	0.84	0.84	0.99	0.99	0.99	1.00				
14	Cat 2, clone 2	0.98	0.98	0.97	0.98	0.93	0.93	0.94	0.85	0.84	0.99	1.00	0.99	1.00	1.00			

ATCC 700432<sup>T</sup> 16S rRNA gene sequences were identical to the sequence of the corresponding 16S rRNA gene in GenBank.

The similarity values between the cloned sequences and that of the closest genus to *Anaerobiospirillum*, *Succinivibrio dextrinosolvens*, ranged between 0.93 and 0.94, whereas those of *Succinimonas amylolytica* and *Ruminobacter amylophilus* ranged between 0.83 and 0.85. The relationship between *Anaerobiospirillum* 16S rRNA gene sequences obtained from the intestines of cats with ileocolitis in the present study was further demonstrated in the phylogenetic tree (Fig. 4). All cloned sequences obtained from cat 1 and one clone each from cats 2 and 6 clustered with a confidence level of 93% of the bootstrap replicates in a branch related to but separate from that of the reference canine strain *A. succiniciproducens*. Branching of the cloned sequences obtained from clones 1 and 3 from cat 2 with the reference *A. succiniciproducens* strain was supported by 98% of the bootstrap replicates. The reference human *A. thomasi* strain was an outgroup of the above two branches.

## DISCUSSION

On the basis of the ultrastructural morphology of spiral flagellated bacteria consistent with an *Anaerobiospirillum* species and the known association of these microbes with enteric diseases of humans, we hypothesized that an *Anaerobiospirillum* sp. was implicated in the ileocolitis present in these cats. Comparative analysis of partial 16S rRNA gene sequences

obtained from the affected intestinal tissues of three cats revealed an *Anaerobiospirillum* sp. related to but different from the previously cultured canine reference strain *A. succiniciproducens* (2, 10). Cat 1, with severe acute ileitis, and cat 6, with subacute colitis, had *Anaerobiospirillum* sequences that were nearly identical to each other (Table 3; 0.99 to 1.00 similarity values) and more closely related to the reference *A. succiniciproducens* strain than *A. thomasi* strain (Table 3; 0.97 to 0.98 versus 0.92 to 0.93 similarity values). Conversely, cat 2, with subacute colitis, had a mixed population of *Anaerobiospirillum* consisting of two clones that were closely related to the reference canine *A. succiniciproducens* strain and one clone that was similar to that of cats 1 and 6 with ileocolitis. These observations suggest that *Anaerobiospirillum* spp. closely related to but different from *A. succiniciproducens* may be implicated in feline ileocolitis. Additional studies, including genomic analyses of organisms isolated from affected cats, will be required in order to determine the relationship of these organisms to the reference isolates of each species.

The *Anaerobiospirillum* 16S rRNA gene sequence examined in the present study was selected on the basis of unique features distinguishing it from other bacterial lineages (8). Major differences included the absence of a 12-nucleotides region between positions 200 and 215 (*E. coli* nomenclature) in *Anaerobiospirillum* spp. as well as the lack of a 16-nucleotide (*A. thomasi*) and 22-nucleotide (*A. succiniciproducens*) region between positions 452 and 476. These differences accounted for the various lengths of the PCR-amplified 16S rRNA gene

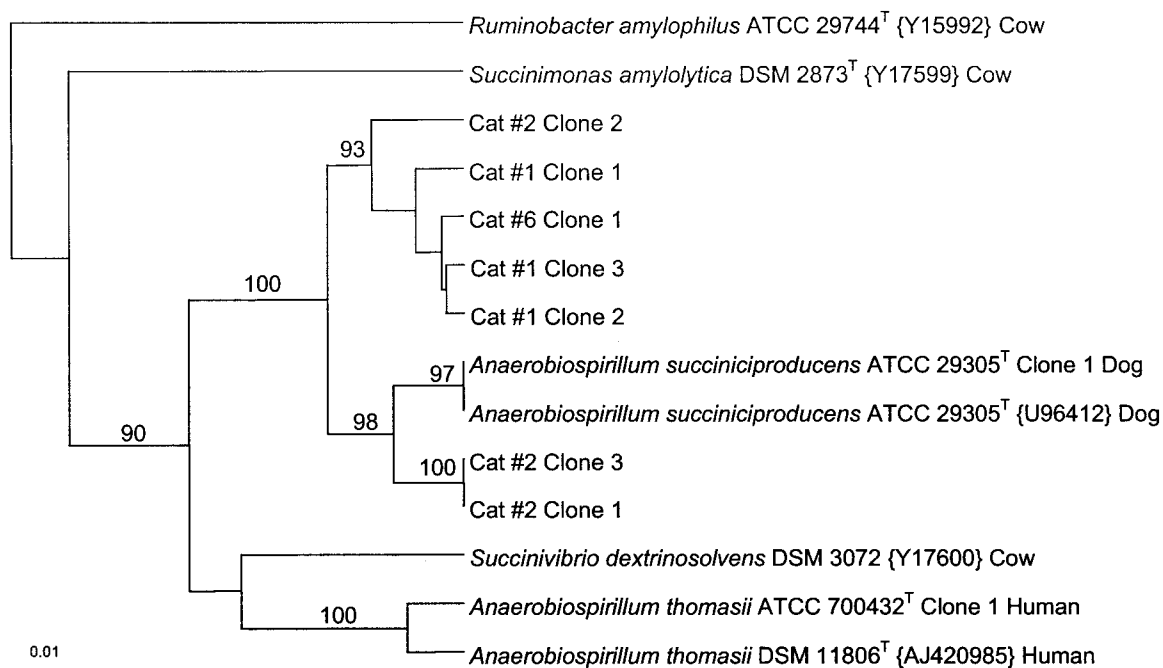


FIG. 4. Phylogenetic tree inferred from partial *Anaerobiospirillum* 16S rRNA gene sequences, indicating that the three clones obtained from cat 1 and one clone each obtained from cats 2 and 6 clustered together in a branch separate from cloned *A. succiniciproducens* reference strain ATCC 29305<sup>T</sup> and the corresponding GenBank sequence (U96412). Conversely, two clones obtained from cat 2 were closely related to reference strains of *A. succiniciproducens*. None of the clones obtained from the affected cats were closely related to the cloned reference *A. thomasi* strain ATCC 700432<sup>T</sup> and corresponding GenBank sequence (AJ420985). As expected, the cloned reference *A. succiniciproducens* ATCC 29305<sup>T</sup> and *A. thomasi* ATCC 700432<sup>T</sup> strain were similar to corresponding sequences retrieved from GenBank. Bootstrap estimates (in percentages) that are higher than 90% of the bootstrap replicates are indicated above the branches. The scale represents 1% expected nucleotide substitutions per site.

sequences that were cloned for analysis, ranging from 335 to 364 bp (Table 2). Also because of these sequence differences, the *Anaerobiospirillum* spp. present in the affected cats were clearly differentiated from the closely related human species *A. thomasi*.

The clinical presentation of these cats was very similar to that described in previous reports of human *Anaerobiospirillum* infections (11, 12, 15). While most patients with diarrhea recover from the infection (11, 13, 15), systemic disease associated with septicemia is a common finding (15). Interestingly, septicemia is most commonly seen in subjects with an underlying disease such as alcoholism, liver failure, cardiomyopathy, lymphoma, or AIDS (15, 22, 23). An immunocompromised state associated with surgery (18) and young age also may represent potential risk factors for septicemic *Anaerobiospirillum* infection in humans (11, 19). In the present series, cats 1 and 2 were adults with cardiomyopathy, whereas cats 3, 4, and 6 were kittens. Additionally, cat 4 became seriously ill postsurgery, whereas cat 3 had a herpesviral infection, a virus strongly associated with immunosuppression in cats (17). Although a specific immunosuppressive condition did not appear to be present in cat 5, this cat clearly had clinical and pathological findings indicative of bacterial septicemia. Interestingly, this cat also had renal failure, a feature that has been reported as a prominent component of *Anaerobiospirillum* septicemia in humans (9, 15, 22). This cat came from a household with 40 other cats, with a history of episodes of diarrhea in the colony. Renal failure was also reported in at least one other cat of the

colony, which died. Since anaerobic blood cultures and PCR amplification of other tissues (spleen, liver, and kidneys) were not performed for any of the cats, the possibility of septicemia in cats with *Anaerobiospirillum* ileocolitis remains speculative.

Intestinal lesions associated with *Anaerobiospirillum* diarrheal disease or septicemia of humans and animals have not been described previously. The cats in the present report had distinct pathological changes restricted to the ileum and/or colon. The observation that *Anaerobiospirillum* bacteria were free within the subepithelial connective tissue in several cats and within the lymphoid follicles of the Peyer's patches of cat 1 supports the possibility that these bacteria are invasive, at least locally, in the gut wall. These observations are consistent with the suggestion that the intestinal tract is the most likely primary site for bacterial entry in septicemia of humans (15). The major differential diagnosis for enterocolitis associated with spiral bacteria in cats is campylobacteriosis. While PCR is the most accurate method to differentiate between *Anaerobiospirillum* and *Campylobacter*, distinct morphological differences between these bacteria confirmed that *Campylobacter* was not the cause of the ileocolitis in the present series of cats (26). While *Anaerobiospirillum* spp. have lophotrichous flagellation, *Campylobacter* spp. display monotrichous flagellation. Additionally, *Anaerobiospirillum* spp. have at least double the cell diameter of *Campylobacter* spp., and the morphology of the flagellar structure of *Anaerobiospirillum* spp. is unique among the microbes present in the intestinal tract (26).

Oral inoculation of a susceptible host with either *A. succi-*



*niciproducens* or *A. thomasi* has not been attempted to date. Consequently, a detailed pathological description of lesions associated with these infections is not available. Therefore, a role for *Anaerobiospirillum* in ileocolitis in general and diarrheal illness in particular, still needs to be confirmed. Controlled challenge infection of susceptible cats with feline *A. succiniciproducens* coupled with detailed sequential sampling of intestinal specimens and thorough pathological investigations are needed to confirm the enteropathogenicity of *Anaerobiospirillum* spp. associated with feline enterocolitis.

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