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Case report:

Septic peritonitis in a dog caused by *Anaerobiospirillum succiniproducens*

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Abstract

This is the first reported case of septic peritonitis caused by *Anaerobiospirillum succiniproducens* in a dog. The infection was associated with marked exfoliation of reactive mesothelial cells into the abdominal fluid mimicking neoplasia. The source of the infection was not determined but was presumed to be of gastrointestinal origin as *A. succiniproducens* is part of the normal gastrointestinal flora of dogs. *Anaerobiospirillum* spp have been previously reported as causing diarrhea and bacteremia in people, particularly if immunocompromised, however there were no indicators for a compromised immune system in this dog.

Key words

Abdominal infection, canine, reactive mesothelial cells, immune compromise,

Case Presentation

An 11-year-old female spayed Labrador Retriever was presented to Bundoora Veterinary Clinic and Hospital with reluctance to stand and walk, abdominal pain, and fever (temperature 40°C/104°F). Abdominal radiographs revealed loss of serosal detail, and a presumptive cranial abdominal mass. Abdominocentesis confirmed the
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33 presence of an abdominal effusion as the cause for the loss of serosal detail.
34 Abdominal ultrasound examination revealed a large amount of anechoic ascites
35 containing small echogenic foci, and in the mid abdomen a soft- tissue and irregular,
36 slightly hyperechoic mass lesion with indistinct margins, which appeared to surround
37 the intestine.

38 Routine hematology revealed an inflammatory leukogram characterized by a marked
39 leukopenia (WBC count $2.1 \times 10^9/L$, reference interval [RI] $4.5\text{--}17.0 \times 10^9/L$) due to a
40 marked neutropenia ($1.3 \times 10^9/L$, RI $3.5\text{--}12.0 \times 10^9/L$) with mild toxic changes, and
41 lymphopenia ($0.6 \times 10^9/L$, RI $0.9\text{--}3.5 \times 10^9/L$). Red blood cell and platelet counts and
42 total protein concentration within the normal range.

43 Abdominal fluid analysis was performed. The fluid was a brown and turbid exudate
44 with a total nucleated cell count (TNCC) of $76 \times 10^9/L$ (RI $<1.5 \times 10^9/L$), and a
45 protein concentration of 35g/L (RI $<25\text{g/L}$). The direct smears were highly cellular
46 containing large numbers of inflammatory cells along with frequent clumps of
47 pleomorphic polygonal cells, and low numbers of erythrocytes in a light
48 proteinaceous background (Figure 1A). The inflammatory cells consisted
49 predominately of degenerate and non-degenerate neutrophils (91%), with occasional
50 lymphocytes (1%) and large mononuclear cells (8%), including vacuolated
51 macrophages. The macrophages often contained blue-green pigment consistent with
52 hemosiderin. The pleomorphic polygonal cells had moderate to abundant basophilic
53 cytoplasm with occasional cytoplasmic ballooning and round to oval nuclei with a
54 lacy to coarse chromatin structure and a prominent nucleolus (Figure 1B). They
55 showed moderate anisokaryosis and marked anisocytosis, occasional binucleation,
56 and some mitoses were noted. Frequent extracellular and occasional intracellular
57 curved or spiraled rod bacteria were identified (Figure 1C). The cytologic
58 interpretation was a septic peritonitis with suspicion of a carcinoma based on the high
59 yield of pleomorphic cohesive cells, with differential diagnoses for these cells
60 representing reactive mesothelial cells or mesothelioma. The morphology of the
61 bacteria was suggestive of *Campylobacter* species, and thus gastrointestinal
62 perforation was suspected.

63 The dog was stabilized with intravenous fluids, antibiotics and analgesics, and then
64 anaesthetized for a midline ventral celiotomy. Extensive peritonitis was confirmed by
65 the presence of a large volume of effusion and a diffusely thickened and inflamed
66 mesentery, with areas of yellow discoloration noted on the colon. The previously

noted mass lesion was identified as an extensive area of firm discolored mesenteric fat and omentum which was adherent to the stomach. The intestinal serosal surface appeared inflamed and thickened with no peristalsis evident. The owner elected to euthanize the dog based on the guarded prognosis.

A necropsy was performed and no perforations or mucosal lesions were identified in the stomach or intestines. Biopsies of the small intestine, stomach, colon, mesentery, and omental mass were evaluated. The mesothelial cells of the mesentery showed moderate hyperplasia (Figure 2A) with multifocal areas of neutrophilic inflammation and fat necrosis. The yellow lesions identified on the colon at surgery were identified as focal areas of neutrophilic inflammation (Figure 2B). No neoplastic tissue was found and no changes indicating a compromised immune system were identified. The organism in the abdominal fluid was confirmed as a Gram negative spiral bacterium. Culture was attempted aerobically on MacConkey agar and horse blood agar at 37°C, anaerobically on nutrient agar at 37°C, and, because of the suspicion that the organism was a *Campylobacter* species, a culture on Campylobacter agar (CAMPY) at 40°C was initiated, however there was no growth in any of these media. The fluid was therefore sent to the Australian Genome Research Facility (AGRF) for identification by 16S RNA sequencing, where the organism was identified as *Anaerobiospirillum succiniproducens* ATCC29305 (Table 1).

Discussion

Anaerobiospirillum succiniproducens is a spiral, motile, gram-negative anaerobic rod bacterium. *Anaerobiospirillum* spp. are part of the normal intestinal flora of cats and dogs¹ and have been reported as a cause of diarrhea and bacteremia in people.^{2,3,4,5} Most reported cases occurred in immunocompromised people, eg in association with lymphoma, alcoholism and liver disease, however an infection has also been reported in a healthy adult man.⁵ In all documented cases the infection was associated with high mortality.

In a survey of 190 dogs and 84 cats *Anaerobiospirillum* spp. was isolated from feces of 27 dogs and 3 cats by microaerobic culture on selective Blaser–Wang's agar plates (Oxoid Ltd., Basingstoke, Hampshire, UK), Skirrow's agar plates (Oxoid) and modified Charcoal Cefoperazone Deoxycholate agar (Oxoid). Some animals were co-infected with *Campylobacter* or *Helicobacter*. Intestinal carriage of

A.succiniproducens was identified in 7.7% of healthy dogs and 7.3% of dogs with diarrhea, and *Anaerobiospirillum thomasi* was identified in 17.3% of healthy dogs and 2.2% of dogs with diarrhea. An unidentified *Anaerobiospirillum* sp. was isolated from one dog with diarrhea.¹ No statistically significant correlation was found between single or multiple infections with *Anaerobiospirillum*, *Campylobacter* or *Helicobacter* sp. and the presence of diarrhea.¹

Infestation with *Anaerobiospirillum* spp. has been associated with hemorrhagic diarrhea in a puppy⁶ and with ileocolitis in cats⁷, however these bacteria were not identical with *A.succiniproducens* or *A.thomasi*, and there was only 93.7% sequence similarity with *A.succiniproducens* in the puppy with diarrhea. In people *A.succiniproducens* is more likely to be associated with bacteremia and diarrhea, whereas *A.thomasi* is implicated as cause of diarrhea but not bacteremia.

This is the first reported case of peritonitis in a dog due to *A.succiniproducens*. The source of the *A.succiniproducens* was not identified in this dog, but was presumed to originate in the gastrointestinal tract. The infection with *A.succiniproducens* in this dog elicited a marked peritoneal inflammation with exfoliation of numerous reactive mesothelial cells mimicking neoplasia. Reactive mesothelial cells can display significant dysplasia in response to inflammation or hemorrhage, including pleomorphism, multinucleation, prominent nucleoli, and mitoses.⁸ In this case the degree of exfoliation of these dysplastic cells was considered unusual and excessive, and suggestive of a carcinoma or mesothelioma rather than reactive hyperplasia, however an extensive post mortem examination failed to identify neoplastic tissue and biopsies confirmed mesothelial hyperplasia.

Anaerobiospirillum spp. can be erroneously identified as *Campylobacter* based on their similar morphology, motility and negative gram staining. Culture can assist with differentiation, with *Campylobacter* being positive for oxidase and catalase activity, and *Anaerobiospirillum* spp. negative for both oxidase and catalase. Morphologically, *Anaerobiospirillum* spp. have bipolar tufts of flagella, whereas *Campylobacter* has a single flagellum at one or both poles.⁴ *A.succiniproducens* is often resistant to macrolide antibiotics, and the optimal therapy for dogs and people is not known.⁵ The organism has been reported as sensitive to cefotaxamine, imipenem, sulfabactam/ampicillin, amoxicillin-clavulanic acid and penicillin, and intermediately resistant to metronidazole and clindamycin.^{4,5} The unidentified *Anaerobiospirillum*

spp. in the puppy with diarrhea was reported as susceptible to amoxicillin and cephalothin.⁶

The failure to culture the *A.succiniproducens* bacteria from this case of peritonitis precluded antibiotic sensitivity testing. The cause of the culture failure was not determined. Optimal growth conditions for *Anaerobiospirillum succiniproducens* are anaerobic at 32-37°C.⁹ The organism is very sensitive to the presence of oxygen, and exposure of the abdominal fluid to air after collection may have been a contributing factor to the failure of growth in anaerobic culture.

This is the first reported case of septic peritonitis caused by *A.succiniproducens* in a dog. Recognition of this organism as a potential pathogen in dogs is important given the morphologic similarities to *Campylobacter* species, but differing culture requirements and antibiotic sensitivity.

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Rank	Sequence Entry	E value	% Identity	Hit Length	Gaps

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1	NR_026075.1 <i>Anaerobiospirillum succiniproducens</i> str.S411;ATCC29305	0.0	100%	515	0
2	U96412.1 <i>Anaerobiospirillum succiniproducens</i> str. ATCC29305	0.0	100%	515	0
3	EU863654.1 <i>Anaerobiospirillum succiniproducens</i> str. 6626	0.0	99.81%	515	0
4	Y17600.1 <i>Succinovibrio dextrinosolvens</i> str.DSM3072	0.0	93.55%	512	2
5	AB042819.1 <i>Thiothrix eikelboomii</i> str. AP3;ATCC49788	1e-130	90.41%	386	2

Table 1. Sequence homology of 16S microbial RNA of the isolated organism from a dog with peritonitis in a screen with published *Anaerobiospirillum* sp. sequences at the Australian Genome Research Facility Ltd.

Figure 1. Direct smear of abdominal fluid from a dog with peritonitis due to an infection with *Anaerobiospirillum succiniproducens*, May Grünwald Giemsa stain. (A) The fluid is highly cellular containing predominately neutrophils with lesser numbers of vacuolated macrophages, and frequent clumps of moderately pleomorphic polygonal cells in a proteinaceous background containing blood. x20 objective. (B). The cell clumps contain polygonal cells with moderate amounts of deeply basophilic cytoplasm and oval nuclei of lacy chromatin with multiple prominent nucleoli. The cells display moderate anisokaryosis and marked anisocytosis with a variable nuclear

199 to cytoplasmic ratio. Occasional cells contain blue-black pigment consistent with
200 hemosiderin, which is also evident in the vacuolated macrophages. Neutrophils are
201 present in the background and amongst the cell clumps, along with a few erythrocytes
202 and lysed cells. x100 objective. (C). Besides numerous degenerate neutrophils, 2
203 large mononuclear cells and one macrophage there are spiraled or curved bacteria
204 (thin arrow) and low numbers of erythrocytes in the background. One neutrophil
205 contains a phagocytized a bacterium (thick arrow). x100 objective.

206
207
208 Figure 2. Histologic sections of the mesentery of a dog with peritonitis due to an
209 infection with *Anaerobiospirillum succiniproducens*., H&E stain. x50 objective. (A)
210 The mesothelial cells vary from flat to large plump cuboidal to columnar cells with
211 increased amounts of eosinophilic cytoplasm and ruffled apical cell border. They
212 show moderate anisokaryosis and anisocytosis. Capillaries appear congested and the
213 lymphatics are mildly dilated. (B) In some areas the surface is ulcerated and the
214 mesentery is expanded by an extensive infiltrate of neutrophils.

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