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Concurrent infection with *Streptococcus equisimilis* and *Leishmania* in a dog

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Leishmaniasis in dogs, a common disease in the Mediterranean basin, is caused by members of *Leishmania donovani* complex, mainly *L. leishmania infantum*,[17] that multiply in cells of the mononuclear phagocyte system.[16] The immune system is important in the pathogenesis of the disease in dogs[18] and human beings.[9,11] In dogs, group C streptococcal infections cause septicemia, bronchopneumonia, and urinary tract infections.[13] The purpose of the present report is to describe concurrent group C (*S. equisimilis*) streptococcal and *Leishmania* infection in a dog.

A 5-year-old female mastiff with mucopurulent conjunctivitis, anorexia, and ataxia of 3 day's duration had elevated blood urea nitrogen concentration (42 mg/dl) and low hematocrit (20%). The dog became hypothermic, with signs of vascular collapse, and was euthanized. Two years earlier, this dog had had epidermal scaling, periocular alopecia, pruritus, conjunctivitis, enlarged popliteal lymph nodes, and increased total serum proteins (12.2 g/dl) due to elevation of the beta-gamma globulin fraction (8.2 g/dl). Bone marrow cytology at that time showed numerous organisms within macrophages, consistent with *Leishmania.* The animal responded to treatment with antimonials.

Immediately after euthanasia, tissue samples were fixed for routine histopathologic analysis. Immunohistochemical evaluation to detect *Leishmania* antigen was done following a previously described technique.[20] The anti-*Leishmania* serum was from naturally infected human beings. An avidin-biotin-peroxidase complex (ABC) method was used to demonstrate amyloid in the kidney.[7] A monoclonal antibody against amyloid A[a] (reference M759) was used. Samples from liver, kidney, spleen, and pericardial fluid were plated on Tryticase soy agar plus 5% sheep blood.[b] Plates were incubated aerobically and anaerobically[c] at 37 C for 24 hours. Further identification was performed by Gram's stain, catalase activity, growth in Baird Parker agar, and biochemical studies. Biochemical identification of the bacteria was done using an automated system.[d]

Serohemorrhagic fluid with fibrin distended the peritoneal cavity. Similar fluid filled the pleural space and distended the pericardial sac, which was focally adhered to the right ventricular epicardium. Thrombi were attached to the left atrioventricular valve (Fig. 1). The spleen and mesenteric, hepatic, and cervical lymph nodes were enlarged. The liver had an accentuated lobular pattern and an infarct in the left lateral lobe (Fig. 2).

Numerous protozoal organisms in macrophages of the spleen, lymph nodes, and liver were identified as amastigotes of *Leishmania* by immunohistochemical techniques (Fig. 3) and electron microscopy. *Leishmania* antigen was demonstrated immunohistochemically in macrophages of these or-

Figure 1. Heart; Left atrioventricular valve; dog with vegetative valvular endocarditis (arrows).

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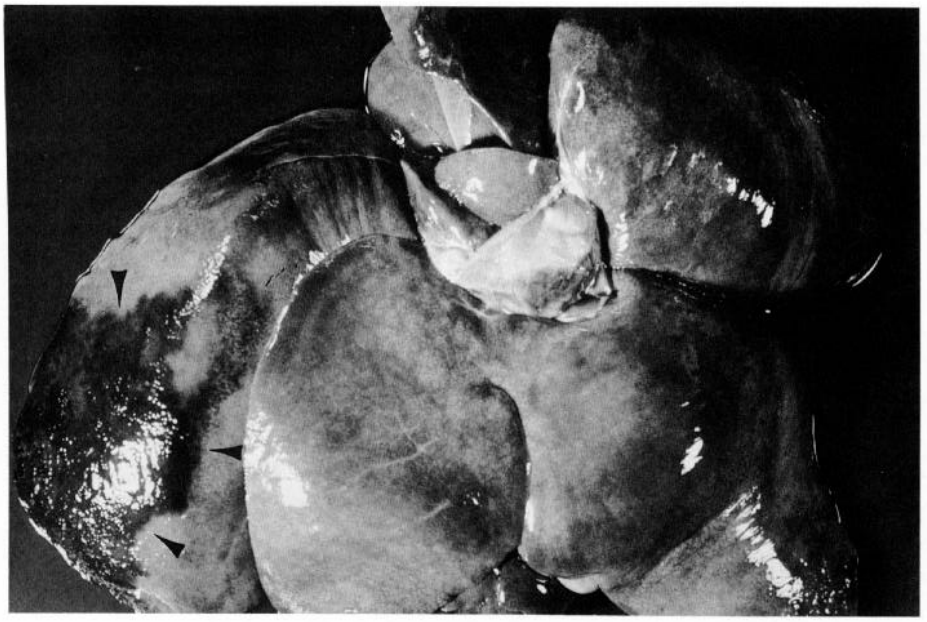

Figure 2. Liver; dog. The dark area in the left lateral lobe (arrowheads) is an infarct.

gans and in small intestinal lamina propria and submucosa and gastric submucosa. Macrophages in these tissues also contained abundant hemosiderin. Gram-positive cocci occurred in hepatic sinusoids, splenic red pulp, lymph nodes, myocardial interstitium, and left atrioventricular valve and in capillaries of the renal glomeruli, small intestinal lamina propria, and brain.

Lymphocytes and plasma cells infiltrated the right ventricular epicardium. Multifocal necrosis and mineralization occurred in the ventricular myocardium, which was infiltrated by neutrophils and lymphocytes. Valvular thrombi contained numerous neutrophils and cocci. Splenic follicles were hyperplastic, and increased numbers of reticulohistiocytic cells occurred in the splenic red pulp. The renal glomerular interstitium was expanded by amyloid deposits (Fig. 4), as confirmed by immunohistochemistry. Electron microscopic examination of glomeruli (Fig. 5) revealed amyloid fibrils in the mesangium and capillary basement membrane,

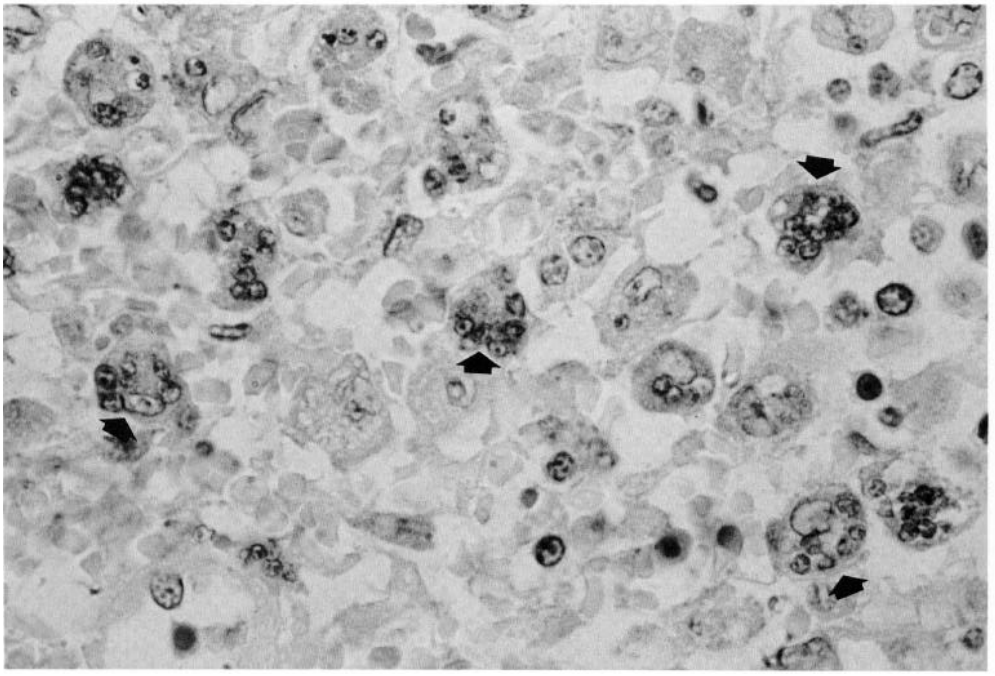

Figure 3. Spleen; dog. Numerous intracytoplasmic *Leishmania* (arrows) are stained with anti-*Leishmania* antiserum. Peroxidase-antiperoxidase, 1,000 ×.

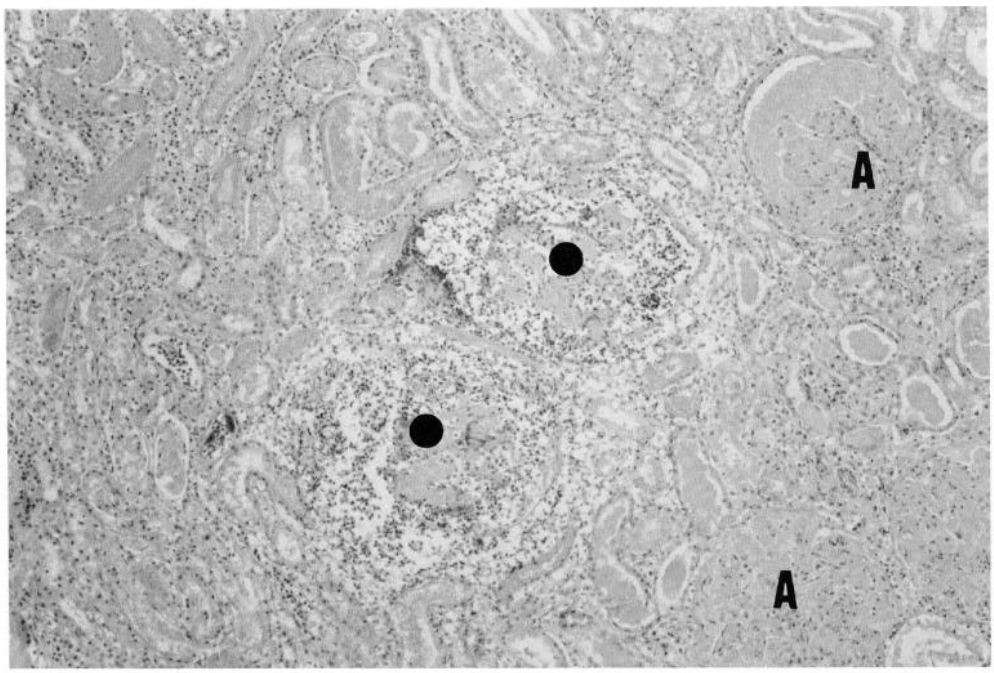

Figure 4. Kidney; dog. Two glomeruli (circle) are almost destroyed and contain suppurative infiltrate. Other glomeruli (A) have amorphous material in the mesangium and proteinaceous material in the Bowman's space. HE, 400 ×.

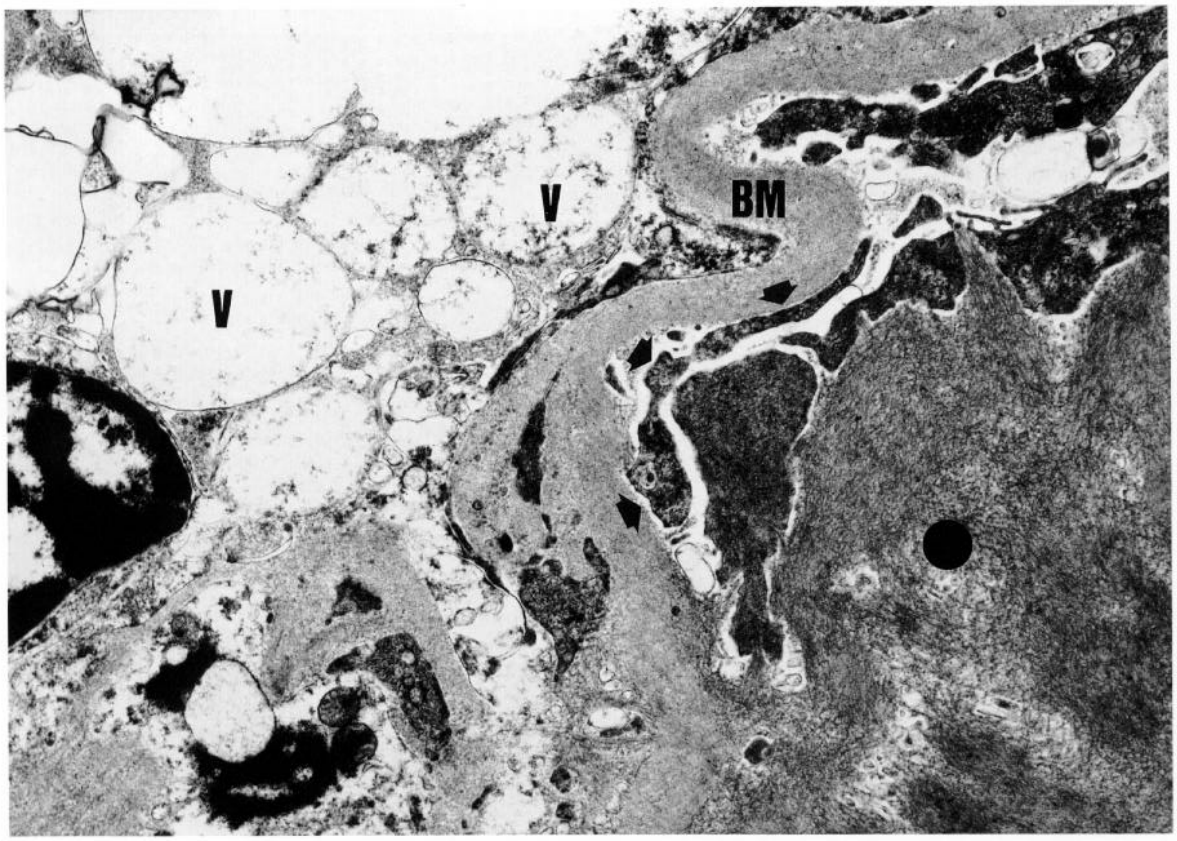

Figure 5. Transmission electron micrograph. Kidney; dog. An extensive deposit of amyloid fibrils arising from the basement membrane fills the Bowman's space (circle). Foot processes of podocytes are fused (arrows) and the cytoplasm of endothelial cells appears severely vacuolized (V). The basement membrane (BM) is folded and segmentally thickened with electron-dense deposits of probable immune complex origin. 13,400 ×.

Table 1. Biochemical characteristics of species of group C *Streptococcus* and isolate FVM5811597.

	β-glucuronidase	Trehalose	Sorbitol	Ribose
S. anginosus	−	+	−	−
S. equisimilis	+	+	−	+
S. equi	+	−	−	−
S. zooepidemicus	+	−	+	+
Isolate FVM5811597	+	+	−	+

beneath endothelium and epithelium, and in the urinary space. Podocyte foot processes were fused and retracted, endothelial cells had vacuolated cytoplasm, and the glomerular basement membrane was folded and thickened. Multifocal necrosis and neutrophil infiltration occurred in gray and white matter of the brain. The leptomeninges were infiltrated with neutrophils and lymphocytes.

A pure culture of ß-hemolytic gram-positive cocci that were catalase-negative was obtained (isolate FVM5811597). No growth in Baird Parker agar was observed. Further typing of the FVM5811597 isolate using biochemical tests led to a final diagnosis of *S. equisimilis* (group C *Streptococcus),* with 99.3% agreement.

Our findings support a diagnosis of septicemic streptococcosis and leishmaniasis. This is the first report of concurrent group C streptococcal (*Streptococcus equisimilis*) and *Leishmania* infection in a dog. The diagnosis of leishmaniasis was based on clinical, pathologic, immunohistologic, and electron microscopic studies.[20] Streptococcal infection was detected by bacteriologic analyses. Group *C Streptococcus* species, which include *S. anginosus, S. zooepidemicus, S. equi,* and *S. equisimilis* as major human and animal pathogens3 are distinguished by their biochemical characteristics (Table l).12 However, three reactions of FVM5811597 isolate were not typical of *S. equisimilis:* the Voges-Proskauer, N-acetyl-ß-glucosaminidase, and alanine-phenylalanine-proline-arylamidase reactions. Some lesions in this dog, such as the reticulohistiocytic hyperplasia of lymphoid organs, plasma cell proliferation in the spleen, hemosiderosis, and glomerular amyloidosis, probably resulted from protozoal infection.[4,10,17,20] Glomerulonephritis with proliferation of mesangial or endothelial cells, or basement membrane thickening,[15] apparently caused by immune complex deposition, is common in canine leishmaniasis.[14] Amyloid deposition is unusual.[17] Amyloidosis in this dog could have resulted from prolonged nonspecific polyclonal hypergammaglobulinemia, which occurs in most infected dogs.[20]

Streptococcus is commonly implicated in canine valvular endocarditis.[6] Although no attempts were made to isolate bacteria from cardiac valves, the presence of numerous gram-positive cocci attached to the thrombi and associated with pyogenic inflammation and the isolation in pure culture from various organs, including the heart, of *S. equisimilis* suggest that the valvular cocci were *S. equisimilis.* Shedding of bacteria from the valve could explain colonization of organs such as the liver and the kidney. Pericardial effusion has been associated with leishmaniasis,[8] but the absence of protozoa by histopathology, the absence of *Leishmania* antigens in the heart (including the epicardium) by immunohistochemistry, and the isolation in pure culture of *S. equisimilis* from the effusion in this dog implicate a bacterial cause.

The relationship of streptococcosis to leishmaniasis in this dog is unclear. Although phagocytic abilities of macrophages from *Leishmania*-infected animals are temporarily altered,[1,5] a recent study[18] demonstrated that immunodepression in *Leishmania*-infected dogs is antigen specific, and affected animals respond positively to other antigens. *Streptococcus equisimilis* septicemia or endocarditis is also uncommon in humans and has been related to underlying diseases, including immunosuppression or neoplasia.[2,19] We did not assess the immune status of this dog, but *Leishmania* parasitism of macrophages could have altered the phagocytic response to other organisms, including bacteria.

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b. Biomerieux España, Madrid, Spain.
c. Anaerocult P, Merck, Germany.
d. ATB system, Biomerieux España, Madrid, Spain.

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Tonsil and turbinate colonization by toxigenic and nontoxigenic strains of *Pasteurella multocida* in conventionally raised swine

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Pneumonia and upper respiratory tract infections, such as atrophic rhinitis, are common and insidious diseases of swine. They are often considered causes of decreased rate of weight gain, inefficient feed conversion, and increased time to market, although these parameters do not absolutely correlate with the severity of lesions. *Pasteurella multocida* is associated with lower and upper respiratory infections, based on results of lung cultures at necropsy and cultures from swabs of the nasal cavity.[4,8,9,15] In the lung, one study showed that nontoxigenic strains were most commonly isolated from acute to subacute pneumonic areas, and toxigenic strains were most commonly isolated from granulomas.[9] In atrophic rhinitis, toxigenic strains are associated with severe, progressive turbinate atrophy.[2-4,8,12] Experimentally, purified toxin induces turbinate atrophy when aerosolized into the nasal cavity or injected into the subcutis, muscle, or peritoneum. 1,6-8,11

Although isolation of *P. multocida* from pneumonic lungs and nasal cavities reflects its etiologic importance, other anatomical locations, such as tonsil, may be a reservoir for *P. multocida* in swine.[2,3,14,15] *Pasteurella multocida* can be isolated from turbinate[6,8] and tonsi1,[2,3,14] however, one study in gnotobiotic pigs and two studies in specific-pathogen-free pigs demonstrated that tonsil is colonized to a greater degree than turbinate.[2,3,13,14] The purpose of this study was to use conventionally reared swine to investigate the relative affinity of *P. multocida* for nasal turbinate mucosa and tonsil and the prevalence of toxigenic strains in a random population of Iowa swine.

Tonsil and turbinate were collected from 53 young and growing swine (20-80 kg) submitted live for necropsy at Iowa State University's Veterinary Diagnostic Laboratory and the National Animal Disease Center (NADC), Ames, Iowa, and from 21 sows killed following routine caesarean sections at the NADC. Tissues were collected (washed and rinsed instruments) and stored frozen at -80 C for up to 2 months. Clinical histories and necropsy findings of pigs from the Diagnostic Laboratory varied greatly, and some pigs had evidence of turbinate atrophy according to visual inspection at necropsy (Table 1). Sows from the NADC were clinically healthy and had no evidence of turbinate atrophy as determined by gross examination.

Thawed tissues were ground in 0.02 M phosphate-buffered saline (pH 7.0, 10% w/w), and 10-fold dilutions were inoculated onto duplicate blood agar (BA) plates (tryptose blood agar base + 5% citrated bovine blood)[a] without or with antimicrobials (Kinyon *P. muhocida* type D protein [KPMD]; plate concentration = bacitracin, 3.75 U/ml; clindamycin, 5 µg/ml; gentamicin, 0.75 µg/ml; amphotericin B, 2.25 µg/ml) to select for *P. multocida.* Suspect colonies on KPMD and additional suspect colonies from BA were subcultured to obtain single colonies on dextrose starch agar[a] and identified by standard methods.[5,15] Tests of over 200 tonsil and turbinate cultures by the Clinical Microbiology Laboratory at Iowa State University demonstrated superior recovery of *P. multocida* from KPMD when compared with BA. In addition, only a small decrease (roughly 10%) in numbers of colony-forming units (CPU) of *P. multocida* were seen in KPMD plates when equal bacterial suspensions were plated onto KPMD and BA. Colonies identified as *P. multocida* were transferred to duplicate nylon membranes and tested for expression of toxin as described with a colony-blot assay.[10] No differences in toxin expression, as determined by the membrane-lift procedure, were seen in bacterial colonies grown on KPMD or BA. *Pasteurella multocida* strains P1683 (toxigenic) and P4214 (nontoxigenic) served as positive and negative controls, respectively, for the colony-blot assay.

Pasteurella multocida was isolated from 35 (66%) of 53

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