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Fatal Case of Endocarditis Associated with *Bartonella henselae* Type I Infection in a Domestic Cat

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We report the first feline case of *Bartonella henselae* endocarditis. Despite negative blood cultures, the cat had high *Bartonella* antibody titers and *B. henselae* type I DNA was detected in the damaged aortic valve. Microscopic examination of the valve revealed endocarditis with small silver positive coccoid structures in endothelial cells.

CASE REPORT

A 6.3-kg, 8-year-old female spayed cat was referred for evaluation of a heart murmur and an arrhythmia detected on physical examination when presented after two episodes of collapse. Thoracic auscultation revealed a grade III/VI systolic murmur and an arrhythmia. Laboratory testing revealed mild hypochromasia (mean corpuscular hemoglobin concentration, 29% [reference range, 30 to 38%]; hemoglobin, 9.2 g/dl [normal range, 9.3 to 15.9 g/dl]). The serum chemistry panel was unremarkable with the exception of a mild hyperglobulinemia (globulin, 5.4 g/dl [reference range, 2.3 to 5.3 g/dl]). Enzyme-linked immunosorbent assays for feline leukemia and feline immunodeficiency virus were negative, and the serum thyroxine level was within normal limits. An electrocardiogram revealed sinus tachycardia with occasional ventricular premature contractions and a left axis deviation (mean electrical axis, 60°; reference range, 0 to 140°). Thoracic radiographs revealed severe cardiomegaly and engorged pulmonary vasculature, although the pulmonary parenchyma was unremarkable. The patient was placed on 1 mg of atenolol/kg of body weight administered orally (p.o.) every 24 h (q24h).

The owner adopted the cat at 3 years of age. The cat was the only cat in the household and was living both indoors and outdoors until the last 2 years. The medical history of the cat was eventless, and the owner usually applied a topical flea preventative during the summer months if she noticed her cat scratching excessively.

When examined by a certified cardiologist, the cat was alert and responsive. Physical examination revealed a grade IV/VI left basilar systolic murmur with no arrhythmias or gallop sounds and a weak femoral pulse. An electrocardiogram indicated a heart rate of 160 and normal sinus rhythm with no evidence of ventricular ectopy. An echocardiogram revealed severe concentric left ventricular hypertrophy with multiple hyperechoic foci noted within the left ventricular free wall (left ventricular free wall thickness, 9.0 mm; interventricular septal thickness, 6.5 mm). The left atrium was severely enlarged (20.5 mm). The aortic valve leaflets were markedly thickened and hyperechoic with restricted systolic excursions. A presumptive diagnosis of valvular endocarditis of the aortic valve with secondary aortic stenosis and insufficiency was made. Further diagnostic tests included serial blood cultures submitted for aerobic and aerobic-anaerobic bacteria and for specific *Bartonella* isolation, as previously described (4, 9). Treatment initiated at this time included orbifloxacin (4 mg/kg p.o. q24h), atenolol (1 mg/kg p.o. q24h), enalapril (0.44 mg/kg p.o. q24h) and aspirin (81 mg p.o. q3days). Serological tests were requested for detection of antibodies against *Ehrlichia canis* and *Ehrlichia risticii*, *Toxoplasma gondii*, *Bartonella* spp., and feline infectious peritonitis virus.

One week after the initiation of therapy, reevaluation of the serum chemistry panel revealed renal parameters and electrolytes to be within normal limits. At this time, all blood culture results were negative. The cat was seronegative for all organisms tested, with the exception of high *Bartonella* titers (for *Bartonella henselae*, 4,096; for *Bartonella clarridgeiae*, 8,192). Doxycycline was added to the antibiotic therapy at 4.5 mg/kg p.o. twice a day (BID).

One month later, the cat was presented for evaluation of dyspnea, and thoracic radiographs revealed moderate pleural effusion. Thoracocentesis yielded 90 ml of serous fluid, and therapy with furosemide (1 mg/kg p.o. BID) was added to the drug regimen. At this time, the *Bartonella*-specific blood culture was still negative. Two days later, the cat was presented again as it was ataxic, hypothermic (36.6°C/98°F), and anorexic. The echocardiogram revealed a critical aortic stenosis, with little movement of the aortic valve leaflets. Despite supportive therapy, the patient experienced cardiopulmonary arrest, and resuscitation was not attempted.

A limited (cardiac) necropsy was authorized and revealed severe concentric left ventricular hypertrophy and severe left atrial-auricular enlargement. The aortic valve leaflets were markedly thickened and opaque with a tan mass located ventral to the left and noncoronary cusps of the aortic valve. A section of the aortic valve and adjacent myocardium was sub-

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mitted for histological examination. The valve was markedly thickened by a proliferation of fibrous tissue within a mucinous stroma, and its base was expanded by a large aggregate of laminated fibrin and neutrophils. The surface of the valve-free portion was irregular with papillary projections. Perivascular infiltration of lymphocytes and plasma cells in the adipose tissue underlying the valve and scattered throughout the myocardium was noted. The mural endothelium was focally hyperplastic, and rare small silver positive coccoid structures were seen in endothelial cells by Warthin-Starry staining. No organisms were identified by transmission electron microscopy in the samples of aortic valve examined.

DNA was extracted from 24 mg of aortic valve tissue and 27 mg of pulmonic valve tissue with the DNeasy Tissue Kit (QIAGEN, Valencia, Calif.) according to the manufacturer's instructions. A PCR assay was conducted for the *gltA* and 16S rRNA genes, as previously described (9, 14). *Bartonella* DNA was amplified from the aortic valve tissue, producing a very intense characteristic 400-bp band for the *gltA* gene fragment and a 1500-bp band for the 16S rRNA gene fragment, while amplification from the pulmonic valve tissue yielded only faint bands in the gel electrophoresis analysis.

The fragment amplified from the *glt*A gene was digested with *Taq*I (Promega, Madison, Wis.), *Hha*I, *Aci*I, and *Mse*I (New England Laboratories, Beverly, Mass.) restriction endonucleases, as recommended by the enzymes' manufacturers (1, 17). The fragment of the 16S rRNA gene was digested with *Dde*I (New England Laboratories). Banding patterns of the digests were compared with *B. henselae* strains (strain U-4 [University of California, Davis] and *B. henselae* Houston-I) and *B. clarridgeiae* (ATCC 51734). Finally, the extracted DNA was sequenced for the *glt*A and 16S rRNA genes, as previously described (4). The FastA program (Wisconsin Sequence Analysis Package, Genetics Computer Group, version 10) was applied first to determine the closest bacterial species or subspecies. Then, the Gap Analysis Program was used for sequence alignments and the determination of the percentage of DNA similarity.

After digestion of the *gltA* gene fragment, the PCR-restriction fragment length polymorphism profile observed was characteristic of *B. henselae*, and the partial sequence was identical to *B. henselae* genotype I (Houston I, ATCC 49882). After digestion of the 16S rRNA gene fragment with the *Dde*I endonuclease, the PCR-restriction fragment length polymorphism profile observed indicated that the fragment was a *B. henselae* genotype I strain. Although a fragment was amplified with the primers for the *Bartonella* 16S rRNA gene, the product was not pure enough for proper whole-sequence analysis. However, the partial sequence was indicative of *B. henselae* type I.

Bartonella spp. are emerging pathogens that have been recognized as causative agents of blood culture-negative endocarditis, mainly in humans (7, 16) and dogs (2, 3, 5). *Bartonella* spp. were first described as a cause of human endocarditis in three separate reports in 1993, one caused by *Bartonella quintana* in an immunocompromised individual (18), one caused by *Bartonella elizabethae* in an immunocompetent patient (6), and the third one caused by *B.* (*Rochalimaea*) *henselae* (10). *Bartonella* species, mainly *B. quintana* and *B. henselae*, account for approximately 3% of human endocarditis cases, with more than 100 human cases reported in the international literature (8, 15, 16). In most human cases of *Bartonella* endocarditis, the vegetative lesions are preferentially located on the aortic valve (15), and patients have high antibody titers (8). Furthermore, most cases of *B. henselae* endocarditis are culture negative but positive by DNA amplification (7). Unfortunately, the genotype of *B. henselae* has never been indicated for any of these patients (16).

In dogs, infective endocarditis is a relatively rare lesion (11). However, several cases of cardiac arrhythmias, endocarditis, or myocarditis have been recently associated with *Bartonella vinsonii* subsp. *berkhoffii* (2, 3), and one case of endocarditis was associated with *B. clarridgeiae* (5).

Bacterial endocarditis has been infrequently reported in cats, and there are only a few reports of vegetative endocarditis in cats (13). *Bartonella* was suggested as a possible etiological agent of endocarditis in two cats, but the authors were not able to confirm the specific *Bartonella* species involved or to demonstrate the presence of *Bartonella* in the lesions observed (13). As Malik et al. mentioned, "the only unequivocal way to confirm a diagnosis of *Bartonella* endocarditis is by demonstrating characteristic organisms in valvular lesions using special stains, culture or the PCR." The present report is therefore the first confirmed evidence of such an association. *B. henselae* DNA was identified mainly in the damaged aortic valve, with only a faint band from the intact pulmonic valve. In most human and dog cases of *Bartonella* endocarditis, the vegetative lesions are also preferentially located on the aortic valve (12, 15, 16). Furthermore, Warthin-Starry staining of a fragment of the aortic valve revealed the presence of silver positive coccoid structures, but no bacteria were visualized by electron microscopy. More likely, this negative result could be associated with the paucity of bacilli in the damaged valve as well as the limited amount of tissue available for testing. Furthermore, the antibiotic treatment initiated on the cat one month prior to its death may have led to the destruction of the limited bacteria present in the damaged valve. The very high antibody titer observed in this cat is also in favor of a *Bartonella* etiology for the endocarditis, as reported in both humans (9) and dogs (12). The lack of isolation of *B. henselae* from the blood of this cat is not surprising, as this bacterium has also rarely been isolated from human cases (2 of 10 cases) of endocarditis (7). This endocarditis case indicates that natural infection of domestic cats with *B. henselae* may have severe health consequences. It also strongly supports further investigations on the chronic effects of long-term *Bartonella* infection in cats.

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