Bartonella-associated inflammatory cardiomyopathy in a dog

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Abstract

A 6-year-old, male, mongrel dog was presented for acute onset of dyspnea and cough. At admission the dog was cachectic and severely depressed. The electrocardiogram showed a sinus rhythm conducted with left bundle truncular branch block, and interrupted by frequent multiform ventricular ectopic beats organized in allorhythmias. Thoracic radiographs revealed a marked cardiomegaly with perihilar edema, while transthoracic echocardiography revealed a dilated cardiomyopathy with segmental dyskinesis. Furosemide, enalapril, pimobendan, and mexiletine were prescribed, and a Holter was scheduled after resolution of congestive heart failure. Three days later the dog died suddenly during sleep. Histopathology revealed diffuse myocyte hypertrophy with multifocal hemorrhages, alternating to areas of severe replacement fibrosis and lympho-plasmocytic infiltrates. Immunohystochemistry stains were strongly positive for T-lymphocyte infiltration (CD3), and weakly positive for B-lymphocytes (CD79). Polymerase chain reaction was positive for *Bartonella spp*. Based on these results, a post-mortem diagnosis of bacterial inflammatory cardiomyopathy was made.

Keywords

Myocarditis, bundle branch block, ventricular arrhythmias.

Abbreviations

cTnI	cardiac troponin I
DCM	dilated cardiomyopathy
EMB	endomyocardial biopsy

Conflicts of interest

The authors do not have any conflicts of interest to disclose.

A 6-year-old, 28-kg, male mongrel dog was referred to our institution for acute onset of dyspnea and coughing. The dog lived outdoors, was current on vaccinations, was receiving monthly heartworm prevention, and had frequent tick infestation. He had a history of idiopathic epilepsy that was managed for the last three years with phenobarbital (3 mg/kg PO q12 h) first and then with imepitoin (10 mg/kg PO q12h). At admission the dog was cachectic and markedly depressed, heart rate was irregular with an average rate of 120 bpm, and a grade II/VI left apical systolic murmur was heard. The respiratory rate was 60 brpm with audible crackles in the caudo-dorsal lung fields. Femoral and dorsal metatarsal pulses were weak with frequent pulse deficits and pulsus alternans.

The 12-lead ECG showed a sinus rhythm with a rate of 120 bpm with wide QRS complexes (100 ms), and a normal axis on the frontal plane (84.45°). The QRS complexes were positive in lead I, II, III, aVF and from V_2 to V_6, with no observable Q waves. The QRS complexes appeared negative in aVR, aVL and V_1 (Figure 1). Frequent multiform ventricular ectopic beats, with a prevalent left bundle branch block morphology often organized in ventricular bigeminy, were also noted. According to the surface electrocardiographic findings, the diagnosis of sinus rhythm conducted with a truncular left bundle branch block and interrupted by frequent ventricular ectopic beats, often organized in allorhythmias, was made.

Thoracic radiographs revealed severe generalized cardiomegaly (vertebral heart score of 14 – normal reference 9.7 $\pm$ 0.5) with pulmonary venous congestion, caudal vena cava dilation, and an interstitial perihilar pulmonary pattern.

Two-dimensional echocardiography showed a dilated cardiomyopathy with segmental

dyskinesis characterized by a dilated left ventricle and left atrium (EDVI 288 ml/m2, left atrial to aortic root dimension ratio 2.4) (Figure 2), and with poor systolic function (ESVI 166 ml/m2, shortening fraction 21%, 2D-based ejection fraction obtained with Simpsons method 23%). Severe left ventricular apical akinesis was noted. A central jet of mitral regurgitation was documented by color Doppler and continuous-wave Doppler (5.43 m/s; pressure gradient 117.9 mmHg). Mild tricuspid regurgitation was also present (3.8 m/s; pressure gradient 57.8 mmHg), suggesting the presence of moderate type II venous passive pulmonary hypertension. No other abnormalities were detected.

A complete blood count revealed mild leukocytosis (white blood cells 19.1 x 10^3/µl, reference range 6.0-17 x 10^3/µl); routine biochemistry revealed mildly elevated AST (126 U/L, reference range 15-45 U/L), ALT (155 U/L, reference range 19-80 U/L), ALP (603 U/L, reference range 15-127) and CK (733 U/l, reference range 42-320 U/L). Serum protein electrophoresis, thyroid profile (tT4 and TSH), and complete coagulation panel (including FDPs and D-dimers) were within reference ranges. Serum cardiac troponin I (cTnI) concentration was approximately 62 times greater than the upper reference range (3.74 ng/ml, reference range <0.06 ng/ml). The antibody screening tests for Anaplasma phagocytophilum, Borrelia burgdorferi and Erlichia canis were negative.[d] Financial constraints prevented the analysis of other possible pathogen titers.

An initial diagnosis of dilated cardiomyopathy secondary to acute myocardial inflammation or acute coronary syndrome with congestive heart failure was made and the dog was treated with furosemide (2 mg/kg PO q8 h), enalapril (0.5 mg/kg PO q12 h), pimobendan (0.25 mg/kg PO q12 h) and mexiletine (6 mg/kg PO q8h).

Twenty-four hours post-admission, thoracic radiographs showed a complete resolution of the pulmonary edema. The dog was discharged; a Holter placement and an endomyocardial biopsy (EMB) were scheduled. Three days later the dog died suddenly

during sleeping.

Owner's consent was granted to perform a post-mortem examination. Gross necropsy of the heart revealed a marked left atrial and ventricular dilation together with multifocal pale regions of grossly visible myocardial necrosis of the left ventricular posterior wall, papillary muscles, interventricular septum, and right ventricular wall. Multiple cardiac samples from the following locations were collected and stored in both 10% buffered formalin and RNAlater buffer for further histopatological and molecular biology examinations, respectively [e]: right atrial free wall, right ventricular free wall, interventricular septum, left atrial free wall, left ventricular free wall. Serial sections (4-µm thick) of samples fixed in 10% buffered formalin were stained by a standard panel of histological and histochemical stains and reactions, including haematoxylin and eosin (H&E), Masson's trichrome for fibrous tissue, and red elastic picrosirius for collagen and elastic fibers. In addition, anti-CD3 and anti-CD79 stains for T- and B-lymphocytes, respectively, were performed on paraffin-embedded tissue sections.

All histopathologic sections revealed severe multifocal replacement fibrosis mixed with lympho-plasmacellular infiltrate, multifocal hemosiderosis, hemorrhage, and cardiomyocyte hypertrophy (Figure 3 A,B,C). Immunohistochemical stains were strongly positive for T-lymphocytes and weakly positive for B-lymphocytes (Figure 4).

The DNA and RNA extractions were optimized for small tissue samples. The biopsy specimens were stored and stabilized in RNAlater buffer. The tissue pieces were disrupted using Tissue Lyser homogenizer[e] and extracted with TRIzol[f] according to the manufacturer's instructions. Afterwards, nucleic acids were quantified and checked for quality by NanoDrop 2000[g] and stored at -80°C. Extracted samples were checked for the absence of PCR inhibitors by amplifying glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (DNA template) and ATPase α subunit transcripts in order to assess RNA

quality and quantity with primers already described. [1] The extracted nuclei acids were submitted to 9 PCR/RT-PCR for the identification of *Canine Enteric Coronavirus, Canine Respiratory Coronavirus, Canine Herpesvirus, Distemper virus, Canine Adenovirus 1, Canine Adenovirus 2, Canine Parvovirus, West Nile Virus and Bartonella spp.* Polymerase chain reaction was positive for *Bartonella spp.*, and negative for other viruses (Figure 5).

According to histopathologic, immunohistochemistry and molecular biology results, a tentative diagnosis of inflammatory cardiomyopathy due to Bartonella *spp.* infection was made.

Discussion

Based on physical, radiographic, electrocardiographic, and echocardiographic findings, differential diagnoses for this case included inflammatory cardiomyopathy, ischemic cardiomyopathy secondary to acute coronary syndrome, arrhythmogenic right ventricular cardiomyopathy, and dilated cardiomyopathy (DCM).

The concomitant presence of left ventricular segmental systolic dysfunction, intraventricular conduction disturbances, and severe increase of cTnI (interpreted as a marker of myocardial cell damage), made the diagnosis of inflammatory cardiomyopathy or ischemic cardiomyopathy more likely. [2-4]

Myocarditis is an inflammatory disease of the heart muscle, diagnosed by established histological, immunological, and immunohistochemical criteria, whereas the term inflammatory cardiomyopathy has been proposed when myocarditis is present in association with systolic dysfunction. [5]

Myocarditis is caused by different infectious and noninfectious stimuli.[3] In humans, it results frequently from viral infections and/or post-viral immune-mediated responses.[3] The spectrum of most commonly detected viruses shifts from classic enteroviruses and

adenovirus to parvovirus B19 and human herpesvirus 6. Furthermore, myocarditis can be triggered by non-viral infections such as *Borrelia burgdorferi* (Lyme disease), *Corynebacterium diphteriae,* or *Trypanosoma Cruzi* (Chagas disease), autoimmune disease, or hypersensitivity/toxic reactions to drugs or toxicosis.[3]

Causes of myocarditis in dogs are similar to humans, although much less is known about the real prevalence of these etiological factors, and ante mortem diagnosis is often confounded due to the lack of definitive diagnostic tests. Reported causes for canine myocarditis include viruses (e.g. Parvovirus, West Nile Virus), protozoal agents (i.e. Trypanosoma Cruzi, Toxoplasma, Hepatozoon, Babesia), bacteria (i.e. Staphylococcus, Streptococcus, Citrobacter, Bartonella, Borrelia), fungal agents (i.e. Coccidioides, Cryptococcus, Aspergillus), helminthes (Toxocara), and noninfectious factors such as autoimmune reactions, toxins, trauma, heat stroke, and hemodynamic shock. [6]

Murine models of enteroviral myocarditis suggest that the course of myocarditis is characterized by 3 phases.[3] The acute phase of myocarditis takes only a few days: viral entry and replication induces acute injury to the myocytes, leading to myocyte necrosis; subsequent exposure of intracellular antigens is followed by activation of the host's immune system, which is characterized by the invasion of natural killer cells and macrophages, followed by T lymphocytes.[3] The subacute phase of myocarditis, which may occur a few weeks to several months after the initial infection, is characterized by myocardial inflammation mediated by the effector cells of the immune system (cytotoxic T-lymphocytes, natural killer cells, macrophages), by loco-regional effect of inflammatory mediators released by the infiltrating lymphocytes, macrophages or endothelial cells, and by direct interaction of the antibodies against myocardial cells.[3,7] The chronic phase of myocarditis is then characterized by multifocal myocardial replacement fibrosis, systolic dysfunction, and development of a DCM with segmental dyskinesis (i.e. inflammatory

cardiomyopathy DCM). [3]

In human patients with biopsy-proven viral infection, left ventricular function has been demonstrated to decrease with persisting cardiac viral genomes, while clearance of viral genomes is associated with a significant improvement in left ventricular ejection fraction. [8] However, in some murine models, autoimmune processes persist independently of the detection of virus genome in the myocardium and lead to the chronic phase, which is characterized by myocardial remodeling and development of DCM. [9] In dogs autoimmune and inflammatory cardiomyopathy have also been described in non viral infections such in case of Chagas' disease. [10]

Considering the segmental systolic dysfunction revealed in this dog, ischemic cardiomyopathy secondary to acute coronary syndrome due to arteriosclerosis or atherosclerosis was also considered as differential diagnosis. Arteriosclerosis is characterized by intimal thickening of coronary arterial wall due to hyaline material deposition, proliferation of smooth muscle cells and fibrocytes. This pathologic process causes a progressive occlusion of the vessel lumen, and a relatively high prevalence of this alteration has been reported in dogs with congestive heart failure. [11] Despite this occurrence, infarcts in dogs are very rarely caused by atherosclerosis, as opposed to human beings.[12] Spontaneous atherosclerosis has been reported in dogs mainly in association with secondary hypercholesterolaemia due to endocrinopathies (primarly hypothyroidism and diabetes mellitus).[13]

The absence of abnormalities found in the coagulation panel and thyroid profile made ischemic cardiomyopathy less likely, although a coronary catheterization and angiogram was necessary to definitively rule out this disease process.

The dog herein reported presented with a truncular left bundle branch block with concomitant frequent ectopic ventricular activity. These electrocardiographic findings are

common in human patients with myocarditis, where nonspecific T-wave and ST-segment changes, together with atrial, nodal, or ventricular conduction abnormalities often associated with supraventricular and ventricular arrhythmias, have been documented.[2] In particular, acute myocarditis has been demonstrated in people to cause transient atrioventricular and intraventricular blocks, secondary to interstitial edema that resolves during the convalescent stage. [14]

In dogs, reports describing complete atrioventricular block and conduction abnormalities associated with myocarditis are present.[15-16] The truncular left bundle branch block found in this dog was highly suggestive of a complex heart lesion causing myocardial necrosis, inflammatory edema, and complete interruptions of conduction through the His bundle and bundle branches.[17] The profound degree of myocardial injury documented at necropsy supported this hypothesis. Left bundle branch block with a prolonged QRS duration of ≥ 120 ms and the presence of ventricular ectopic beats are reported in human medicine to be independent predictors for cardiac death or heart transplantation.[18] In dogs, left bundle brunch block with advanced hypokinetic cardiomyopathy is also associated with increased mortality risk.[19] Because of the frequent ventricular arrhythmias noted in this case, mexiletine was prescribed in order to preserve systolic dysfunction while resolving acute cardiac failure. Unfortunately, the dog died before a Holter could be performed, so detailed information about the quality of the arrhythmia throughout a 24 hour period, as well as response to treatment, are missing.

In this patient, the elevation of cTnI was interpreted as an indicator of severe myocardial injury, a finding that was consistent with the necropsy results. cTnI has been recognized as the most sensitive and specific marker of myocardial cell necrosis in humans, and increased concentrations also have been associated with myocarditis.[4] The normal range of plasma cTnI in dogs is similar to that established for humans and results from a

study in dogs suggesting that plasma concentrations of cTnI may be correlated with degree of myocardial injury.[20-21]

Although the suspicion for subacute/chronic myocarditis was high, the main limit in this case was the difficulty in obtaining an exact antemortem etiologic diagnosis. Frequent tick infestation and leukocytosis make protozoal and/or bacterial myocarditis more likely, but an endomyocardial biopsy (EMB) was necessary to detect the specific pathogen and determine the proper treatment protocol.

Current American Heart Association guidelines for the treatment of heart failure describe EMB as a Class IIa recommendation. [22] Referring to these guidelines, EMB is generally reserved for patients with rapidly progressive clinical heart failure or worsening ventricular dysfunction that persists despite appropriate medical therapy, for patients suspected of having myocardial infiltrative processes, or with rapidly progressive and unexplained cardiomyopathy.

To the authors' knowledge, there are no data regarding indications and utility of EMB in dogs; however, our patient, considering human guidelines, did meet the requirements to justify an EMB.

In human medicine, according to the Dallas criteria, acute myocarditis is defined as histologic evidence of lymphocytic infiltrates in association with myocyte necrosis, while borderline myocarditis is characterized by inflammatory infiltrates without evidence of myocyte necrosis.[23] Chronic myocarditis is characterized histologically by mononuclear cell infiltrates, aggregated interstitial fibrosis and fatty infiltration.[24] The Dallas criteria are limited by the high inter-observer variability in interpreting biopsy specimens, and because non-cellular inflammatory processes cannot be detected.[23] Despite the considerable limitations of EMB findings, EMB remain the gold standard for unequivocally establishing the diagnosis. Additionally, with the introduction of immunohistological methods, the

number of EMB revealing myocarditis has markedly increased. [3,9] According to the WHO/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, EMB are considered to be positive for myocarditis by immunohistochemical detection of focal or diffuse mononuclear infiltrates (T lymphocytes and macrophages) with >14 cells/mm^2.[25] The identification of the causative agents of myocarditis can be performed by the application of molecular biological tools to EMB.[3]

According to the histological criteria described, the multifocal severe T lymphocytic infiltrate observed in our specimens was suggestive of myocarditis.[25] The complementary presence of multifocal severe replacement fibrosis, together with the echocardiographic findings of a DCM with segmental dyskinesis, made the chronic form of myocarditis more likely.

There are some important differences between our specimens and those that are usually obtained with EMB. First, the size of EMB samples is small, sample number is limited, and the samples are taken from the apex of the right ventricle and from the interventricular septum only. With these limitations, there is a risk of missing mild focal myocarditis. In this case, though large samples were obtained during necropsy, considering the multifocal and severe myocardial damage, in vivo EMB would have probably offered similar results. Through PCR analysis, we tested the most common etiologic agents reported to cause myocarditis in dogs [6] and all viruses resulted to be negative. Others rare non viral agents causing myocarditis in dogs could not be tested with molecular biology due to financial constrains.

In this case, although a virus-negative PCR cannot exclude viral disease and the positivity for *Bartonella spp* is not enough to demonstrate it as the primary cause of the disease [25], considering history, clinical and instrumental findings, *Bartonella spp.* infection was the most likely cause of the inflammatory cardiomyopathy.

Important limitations to note were the absence of a parallel investigation on blood samples collected at the time of death, and the lack of differentiation of Bartonella species by the PCR assay. Bartonella PCR blood tests would have ruled out the possibility of passive blood contamination of the myocardium.[25]

Myocarditis associated with Bartonella infection is an emerging disease affecting dogs of any age, with exposure to ticks considered a mode of infection.[26] Bartonella species are gram-negative bacteria that usually cause long-lasting intra-erythrocytic bacteremia in mammalian reservoirs, and *Bartonella vinsonii subsp. Berkhoffi* is the most often isolated species in dogs.[26] The disease exhibits a wide range of clinical signs, due to its endotheliotropic nature and to the redistribution throughout the body inside the erythrocytes and macrophages. *Bartonella vinsonii subsp. Berkhoffi*, similarly to our case, has been reported to cause arrhythmias, endocarditis, myocarditis, syncope, and sudden death in dogs.[26-27]

An exact antemortem etiologic diagnosis with endomyocardial biopsy with tissue PCR in this case would have provided useful indications regarding prognosis and additional treatment, as antibiotics that achieve high intracellular concentrations (e.g. azithromycin, doxycycline, enrofloxacin) are presumably beneficial. However, it should be noted that the antibiotics of choice and the duration of treatment for Bartonellosis have not yet been clearly established in dogs.[27]

In conclusion, this case supports a role for Bartolella spp as causative agents of myocarditis in the dog with unexplained cardiomyopathy, particularly when in association with conduction abnormalities and supraventricular and/or ventricular arrhythmias.

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Figure 1: Twelve-lead surface ECG recordings obtained at admission, showing the presence of sinus rhythm conducted with truncular left bundle branch block, interrupted by frequent ventricular ectopic beats with right bundle block morphology organized in allorhythmias. Paper speed=50 mm/s; 1 cm=1 mV.

Figure 2: Transthoracic echocardiogram recorded from the right parasternal long axis view. A segmental dilated cardiomyopathy phenotype characterized by a dilated left atrium and left ventricle and poor systolic function was documented.

Figure 3: Left ventricular myocardial histopathologic stains. (A) H&E Stain (20 x). Severe replacement fibrosis mixed with *lymphoplasmacellular* infiltrate. (B) H&E Stain (10 x). Severe replacement fibrosis mixed with *lymphoplasmacellular* infiltrate associated with hemorrhage and m*yocytolysis*. (C) *Masson's trichrome* stain (10x) Multifocal severe replacement fibrosis.

Figure 4: Left ventricular myocardial histopathologic stain. (40 X) Immunoistochemical stains positive for CD3 (T-lymphocytes).

Figure 5: PCR 16s-23s intergenic sequences showing the positive result of Bartonella spp. An ethidium bromide-stained agarose gel (2,5%) demonstrating amplified products from cardiac samples. The first and second lines each contain a positive control of B. henselae and B. vinsonii, respectively. The other three lines contain the biopsy samples tested, with the positive sample reported in this report (fifth line). The last line contains a 100bp ladder.