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Pasteurella canis infective endocarditis in a dog

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ABSTRACT

Infective endocarditis, an infrequent clinical syndrome in dogs, is typically associated with nondescript clinical signs such as fever, malaise and loss of appetite. Although an uncommonly reported infection in dogs, *Pasteurella canis* is an emerging pathogen with increasing relevance in the human microbiology literature. The goal of this study is to detail the clinical presentation and microbiological findings associated with a novel causative agent of infective endocarditis in the dog. Diagnostic evaluation as well as conventional, automated and molecular microbiological methods are highlighted. The recent literature regarding *P. canis* and infective endocarditis in companion animals and humans is reviewed. Although an unusual etiologic agent of infective endocarditis, awareness of *P. canis* as a diagnostic possibility is crucial to accurate microbial surveillance.

1. Introduction

Pasteurella spp. are Gram-negative, non-motile, coccobacilli that are commensal in the oral cavities of dogs, and have been isolated from 2.8 to 20% of dog oral cavity specimens, including both healthy dogs and dogs with periodontal disease (Baldrias et al., 1988; Meyers et al., 2008; Riggio et al., 2011). Accordingly, *Pasteurella* spp. are often isolated in wounds from animal bites with *P. multocida* subsp. *multocida* being isolated most often. *P. multocida* subsp. *septica*, *P. canis*, *P. dagmatis*, and *P. stomatis* are also found in these wounds (Talan et al., 1999). *Pasteurella canis* has been pathologically associated with superficial infections (pyoderma, cutaneous abscesses) in animals and up to 26% of dog-inflicted bites in humans (Talan et al., 1999; Hara et al., 2002; Kim et al., 2016). Isolated reports of systemic, occasionally fatal, infections caused by *P. canis* are also described in the human literature (Hara et al., 2002; Albert and Stevens, 2010; Hazelton et al., 2013; Bhat et al., 2015).

In the present study, *P. canis* was isolated from the blood and heart valve of an adult dog for the first time. The results highlight an additional species of *Pasteurella* that should be considered when evaluating for causes of bacteremia and infective endocarditis in dogs. The identification of *P. canis* in this infection also highlights an intersection of risk in companion animal and human health.

2. Materials and methods

2.1. Clinical presentation

A 5-year old sexually intact male English Springer Spaniel was presented to its primary veterinarian for multiple superficial dermal abrasions and lacerations incurred during a recent hunting trip in Texas. Owner-initiated empirical treatments included a single dose of carprofen (dosage not specified) and cephalexin (dosage not specified), administered the previous day. Physical examination findings included a partial-thickness right corneal ulcer, distal right thoracic limb swelling, tongue swelling and oral pain. Pertinent hematological (ProCyte Dx, IDEXX Laboratories, Inc., Westbrook, MN) and serum biochemistry (Catalyst Dx, IDEXX Laboratories, Inc.) findings from automated benchtop analyzers included monocytosis (3.41 k/μL, reference interval [RI]: 0.16–1.12 k/μL), neutrophilia (12.56 k/μL, RI: 2.95–11.64 k/μL), thrombocytopenia (79 k/μL, RI: 148–484 k/μL), increased serum alkaline phosphatase (ALP) activity (328 U/L, RI: 23–212 U/L) and serum alanine aminotransferase (ALT) activity (134 U/L, RI: 10–125 U/L). During the 4-day hospitalization period, the dog was treated empirically with intravenous fluid therapy, clindamycin (150 mg PO q12 h), carprofen (37.5 mg PO q12 h), ciprofloxacin 0.3% ophthalmic solution (OD q6h), atropine eye drops (OD q24 h), and a dose of fluralaner (Bravecto, Merck Animal Health; Madison, NJ). Thoracic and

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abdominal radiographs were unremarkable. A fever of unknown origin polymerase chain reaction (PCR) panel (IDEXX Laboratories, Inc.) was negative for amplification of *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Histoplasma capsulatum*, *Brucella canis*, *Bartonella* spp., *Cryptococcus* spp., *Toxoplasma gondii*, *Blastomyces dermatitidis*, *Coccidioides* spp., *Neospora caninum*, *Leishmania* spp., *Leptospira* spp., and *Trypanosoma cruzi* DNA. Doxycycline (100 mg PO q12 h) was added to the treatment plan after the first day of hospitalization. Serial complete blood counts (CBC), performed both on benchtop analyzers and through a major veterinary diagnostic laboratory (IDEXX Laboratories, Inc.) with cytologist review, documented persistent thrombocytopenia and the development of a microcytic, normochromic, nonregenerative anemia (hematocrit [Hct] 35.2%, RI: 37.3–61.7%; hemoglobin [Hgb] 13.0 g/dL, RI: 13.1–20.5 g/dL; mean corpuscular hemoglobin concentration [MCHC] 36.9 g/dL, RI: 32.0–37.9 g/dL; mean corpuscular volume [MCV] 60.8 fL, RI: 61.6–73.5 fL). Serial clinicopathological values of interest are provided in supplemental table 1.

When the dog's clinical condition failed to improve despite therapy, it was referred to the North Carolina State University Veterinary Hospital (NCSU-VH). The dog lived in a facility with 60 other hunting dogs, received routine flea and tick prophylaxis, and was current on core vaccinations (rabies virus, canine distemper virus, canine adenovirus-2, parainfluenza virus, and canine parvovirus) and a multivalent *Leptospira* spp. vaccination. The dog's medical history prior to presentation was unremarkable. Vital parameters were normal (temperature 38.2 °C (100.8 °F), pulse 108 beats per minute, respiratory rate 30 beats per minute). Physical examination abnormalities included a tongue ulcer; a 5 mm ovoid corneal ulcer involving the right eye, approximately 80% of corneal depth with aqueous flare and fibrin admixed with hypopyon on the endothelial surface of the ulcer; mild right mandibular lymphadenomegaly; and multifocal erosions and ulcerations of the dermal pads and scrotum. A heart murmur was not auscultated. Hematology and serum biochemistry were consistent with previous analyses. A lateral flow immunochromatographic enzyme-linked immunosorbent assay (ELISA; SNAP 4Dx Plus, IDEXX Laboratories, Inc.) was negative for *Dirofilaria immitis, Borrelia burgdorferi*, *Ehrlichia* spp., and *Anaplasma* spp. Coagulation abnormalities included a prolonged prothrombin (11.5 s, RI: 7.6–9.4 s) and activated partial thromboplastin (13.4 s, RI: 10.6–13.2 s) times, thrombocytopenia (108×10^3/μL) and hyperfibrinogenemia (302 mg/dL, RI: 100–272 mg/dL); D-dimers and thromboelastography were normal. Right carpus radiographs revealed moderate soft tissue swelling with no associated osseous lesions. Right carpal synovial fluid cytology identified moderately cellular suppurative inflammation, composed of non-degenerate neutrophils with no etiologic agents identified; aerobic culture of the joint fluid yielded no growth. By aspiration cytology, the right mandibular lymph node contained reactive lymphocytes. Thoracic radiographs were unremarkable. Abdominal ultrasonography was unremarkable except for mild diffuse hepatic hyperechogenicity.

The dog was hospitalized and treated for vasculitis, the presumed cause for the multifocal dermatologic lesions, tongue ulceration, and consumptive thrombocytopenia. Clindamycin (175 mg PO q12 h) and doxycycline (75 mg PO q12 h) administration was continued. Topical ofloxacin, autologous serum, and atropine were instilled into the right eye for stabilization, and then treated definitively for a presumed anterior chamber foreign body with anterior chamber lavage and dorsal conjunctival pedicle graft. Following ocular surgery, carprofen (37.5 mg PO q12 h) was administered for 3 days for analgesia and to minimize inflammation. By hospital day 3, anorexia and lethargy had resolved, and the anemia and thrombocytopenia were improved. The dog was discharged for continued monitoring at home and continuation of the same antibiotic regimen for an additional 21 days.

Three weeks later, the dog became acutely lethargic, anorexic and tachypneic and had a kyphotic stance. Rectal temperature was 40.2 °C (104.3 °F). Other physical examination findings included ulceration of the right side of the tongue, increased lung sounds, pain on abdominal palpation, an effusive and warm right carpus, and shifting pelvic limb lameness, worse on the right side. Pertinent findings from in-house hematology included a normochromic, microcytic, nonregenerative anemia (Hgb 11.4 g/dL, Hct 31.5%, MCV 60.6 fL, MCH 21.9 pg), and marked thrombocytopenia (6×10^3/μL). Benchtop serum chemistry revealed increased blood urea nitrogen ([BUN] 47 mg/dL, RI: 7–27 mg/dL) and hypoalbuminemia (2.1 g/dL). Other previously noted abnormalities had resolved. Hepatomegaly and splenomegaly were visualized on lateral abdominal radiographs. Thoracic radiographs were unremarkable. The dog was referred to the NCSU-VH.

At presentation, rectal temperature had normalized. Physical examination findings included gingival petechiae, a healing conjunctival graft, overall reluctance to walk, moderate right carpal effusion, and dull mentation. Thrombocytopenia, estimated at 12×10^3/μL on an automated count, was confirmed by blood smear examination. Vincristine (0.02 mg/kg IV once), dexamethasone SP (0.15 mg/kg IV q24 h), intravenous fluid therapy, pantoprazole (1 mg/kg IV q12 h), and sucralfate (1 g PO q6h) were administered for suspected gastrointestinal hemorrhage secondary to presumptive immune-mediated thrombocytopenia.

Hematological abnormalities the following morning included a progressive normocytic, normochromic, nonregenerative anemia (Hct 28.0%, Hgb 9.4 g/dL, MCV 66.5 fL, MCH 22.3 pg, reticulocytes 40,000/μL); marked neutrophilic leukocytosis with a left shift (WBC 23.42×10^3/μL, segmented neutrophils 21.078×10^3/μL, band neutrophils 0.468×10^3/μL); and thrombocytopenia (15×10^3/μL). Serum biochemical abnormalities included increased BUN (56 mg/dL, RI: 6 – 26 mg/dL), hypoalbuminemia (1.5 g/dL, RI: 3.0–3.9 g/dL) and hypoproteinemia (4.1 g/dL, RI: 5.2–7.3 g/dL). A new grade IV/VI systolic heart murmur, with point of maximal intensity over the left apex, was auscultated. Given the severe inflammatory leukogram and newly auscultated murmur, vegetative endocarditis was suspected. Transthoracic echocardiographic findings included normal right-sided chamber sizes, normal aortic, pulmonic, and tricuspid valve morphology, and normal systolic and diastolic function. The left ventricle and left atrium were mildly dilated and a large, mobile vegetative lesion was identified on the anterior mitral valve leaflet with resultant mitral valve regurgitation (Fig. 1). Blood samples were acquired for automated blood culture, vector-borne disease testing, and *Bartonella* alpha proteobacteria growth medium (BAPGM; Galaxy Diagnostics, Morrisville, NC) enrichment blood culture/PCR. Due to the guarded prognosis for a full and functional recovery, the owners subsequently elected humane euthanasia.

2.2. Postmortem examination

The dog was submitted for postmortem examination. Evaluation of the heart revealed a focal red-tan, proliferative, vegetative lesion adhered to a 2.0 x 0.5 cm segment of the anterior leaflet of the mitral valve. The vegetative lesion was "cauliflower-like" with multiple 1–3 mm, irregular, fibrinous nodules (Fig. 2A). The adjacent and underlying valve surface was roughened and granular. The left atrium was mildly dilated. Additional findings included ulcerative lesions involving the tongue, multiple paw pads, and gastric mucosa. The tongue had a $1.2 \times 0.4 \times 0.4$ cm irregular region of parenchymal loss with a granular, red ulcerated rim. Ulcerations were present on multiple thoracic and pelvic limb paw pads and ranged in size from pinpoint to 3 mm. The stomach contained multiple linear superficial ulcerations within the fundic and pyloric regions. The largest ulceration was located in the fundus and measured 3.0×0.5 cm. The liver was diffusely enlarged, weighing 7.04% of total body weight (RI: 3–4%).

Sections of mitral valve, myocardium, tongue, stomach, liver, and paw pads were processed routinely and stained with hematoxylin and eosin for histologic examination. The anterior leaflet of the mitral valve was markedly disrupted and replaced by degenerative neutrophils,

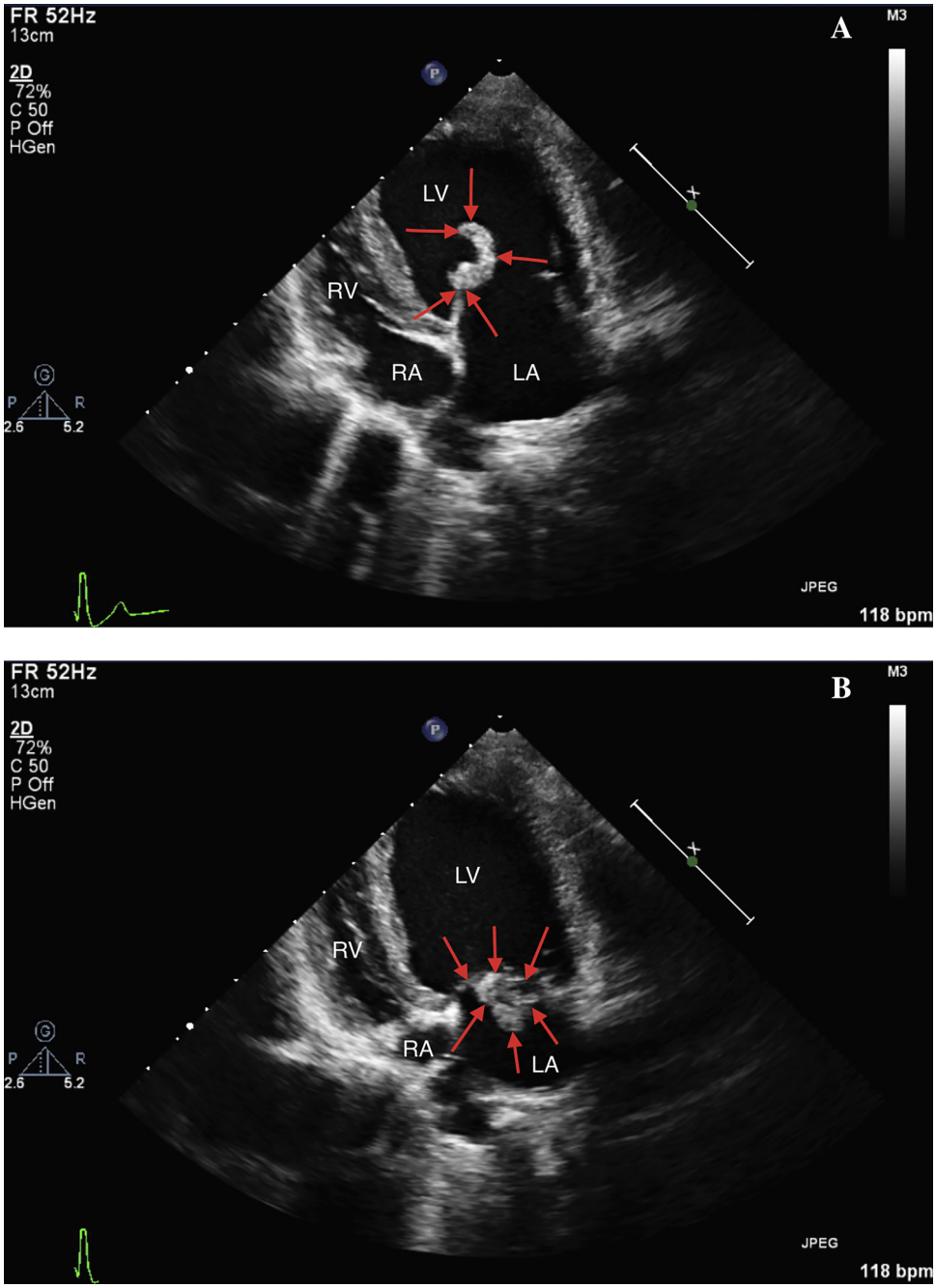

Fig. 1. Transthoracic echocardiogram (left apical 4-chamber view). Vegetative lesion (red arrows) on the anterior leaflet of the mitral valve in diastole (A) and systole (B). Abbreviations: LV – left ventricle, LA – left atrium, RV – right ventricle, RA – right atrium (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

histiocytes, and multinucleated giant cells intermixed with fibrin, hemorrhage, edema, and necrotic cellular and nuclear debris. The surface of the ulcerated valve was overlain by abundant layers of fibrin and myriad large colonies of coccobacilli with occasional foci of hemorrhage and mineralization (Fig. 2B). A Gram stain identified clusters of Gram-negative coccobacilli. The most severely affected ulcerated lesion was the tongue, which had full-thickness mucosal necrosis that extended into and partially replaced the underlying connective tissue. Subjacent to this lesion, was robust granulation tissue without evidence of vasculitis. Based on the clinical data, postmortem gross examination, and histologic findings, a diagnosis of infective endocarditis due to a Gram-negative coccobacillus with presumptive sepsis and consumptive thrombocytopenia was concluded.

2.3. Microbiological analysis

Using an automated blood culture system (Bactec 9050, Becton Dickinson, Franklin Lakes, NJ), broth was processed using standardized microbiological protocols by the NCSU Clinical Microbiology and Molecular Diagnostic Laboratory (CMMDL). *Bartonella* alpha proteobacteria growth medium enrichment blood culture was concurrently performed in the Intracellular Pathogens Research Laboratory as previously described (Duncan et al., 2007; Balakrishnan et al., 2014). Identification and antimicrobial susceptibility testing (ID/AST) were performed using the TREK Sensititre ID/AST system (Thermo Scientific, Oakwood Village, OH) according to manufacturer instructions. *Bartonella* alpha proteobacteria growth medium enrichment blood culture

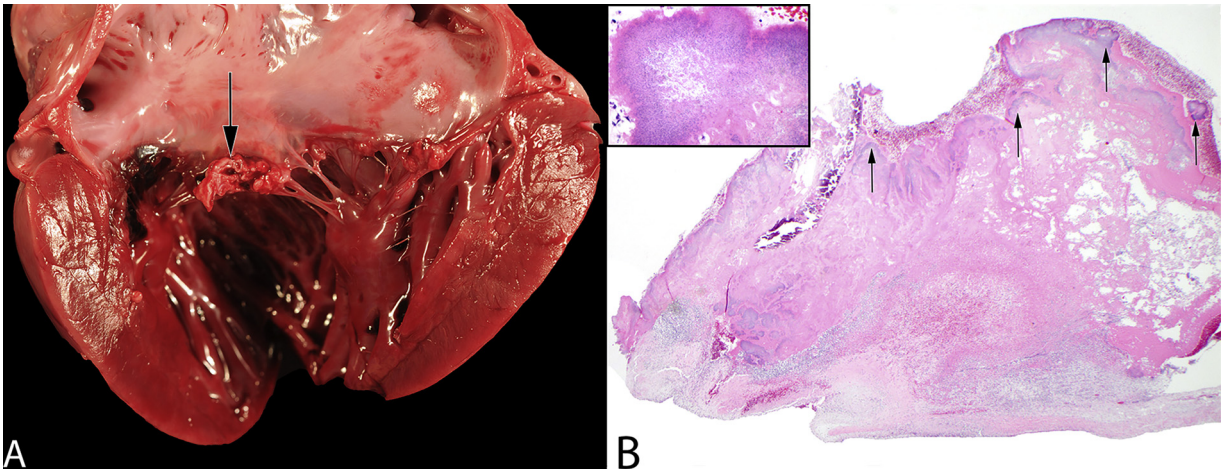

Fig. 2. Gross photograph (A) and photomicrograph (B). A red-tan, friable, vegetative lesion is adhered to the anterior leaflet of the mitral valve (A, black arrow) which is histologically composed of alternating layers of fibrin and abundant myriad coccobacilli (B, black arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

was performed because *Bartonella* spp. are an important cause of both fever and endocarditis in dogs in our region (Davenport et al., 2013), and isolation of these fastidious bacteria using automated blood culture systems is generally unsuccessful (Sykes et al., 2006).

2.4. Molecular analyses

The canine vector borne disease serology and PCR panel includes lateral flow immunochromatographic ELISA (SNAP 4Dx Plus, IDEXX Laboratories, Inc.); *Babesia* spp., *Bartonella* spp., *Ehrlichia* spp. and *Rickettsia rickettsii* immunofluorescence assays (IFA); and *Anaplasma, Babesia, Bartonella, Ehrlichia,* hemotropic *Mycoplasma,* and *Rickettsia* genus PCR assays (Qurollo et al., 2014). *Bartonella* PCR was performed from 7-, 14- and 21-day BAPGM enrichment blood cultures using primers targeting the 16 S–23 S intergenic transcribed spacer (ITS) region as previously described (Balakrishnan et al., 2014).

Following agar plate subculture with bacteria grown from the blood culture bottles, DNA was extracted from the bacterial colonies, and PCR was performed as described previously (Balakrishnan et al., 2016). DNA was also extracted from fresh mitral valve tissue using QIAmp Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Elution buffer was used as a reagent control with each set of DNA extractions. DNA concentration and purity were determined using a spectrophotometer (Nanodrop, Wilmington, DE). Extracted DNA was stored at -20° C until processing.

Eubacterial pan-oligonucleotide PCR targeting the 16S rRNA gene was performed using extracted DNA from the bacterial isolate and fresh heart valve tissue as described previously (Balakrishnan et al., 2016). Chromatogram evaluation and sequence alignment were performed using Contig-Express and Align X software (Vector NTI Suite 10.1, Invitrogen Corp., Carlsbad, CA). Bacterial species and strain were defined by comparing similarities with reference sequences chosen from the GenBank database using Basic Local Alignment Search Tool (BLAST version 2.0).

2.5. Matrix-assisted laser desorption/ionization – time of flight mass spectrometry

The bacterial species was further characterized by matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS; Vitek MS, Biomérieux, St. Louis, MO) according to manufacturer instructions.

3. Results

3.1. Microbiological analysis

Three of three blood culture bottles were flagged as positive by the automated blood culture system. Subsequently, broth that grew bacteria was sub-cultured onto trypticase soy agar II (TSA II) supplemented with 5% sheep blood plates (Becton Dickinson Diagnostic Systems, Sparks, MD). The plates were incubated at 37° C containing 5% CO_2 and 99% relative humidity. After 24 h of incubation, non-hemolytic, grey, smooth colonies were visualized on the blood agar surface, whereas no growth was visualized on MacConkey agar. The organisms were Gram-negative pleomorphic coccobacilli. By TREK Sensititre ID/AST system, the isolate was identified as *Pasteurella multocida* subsp. *multocida* with 94.74% identity and was susceptible to amikacin, amoxicillin/clavulanic acid, ampicillin, cefazolin, cefovecin, cefoxitin, cefpodoxime, cephalothin, chloramphenicol, doxycycline, gentamicin, imipenem, ticarcillin, ticarcillin/clavulanic acid and trimethoprim/sulfamethoxazole. The isolate was resistant to penicillin, erythromycin and clindamycin. BAPGM enrichment 7-, 14- and 21-day blood cultures were negative by both 16 S–23 S *Bartonella* ITS PCR and 16S rDNA eubacterial PCR. Subculture from liquid BAPGM did not result in bacterial growth.

3.2. Molecular analyses

The dog was not seroreactive to *Anaplasma* spp., *Babesia canis*, *Borrelia burgdorferi*, *Bartonella henselae, Bartonella koehlerae, Bartonella vinsonii* subsp. *berkhoffii* or *Ehrlichia* spp. antigens; whereas the *Rickettsia rickettsii* IFA antibody titer was 1:128. The SNAP 4Dx Plus (IDEXX Laboratories, Inc.) *Dirofilaria immitis* antigen assay was also negative. *Anaplasma, Babesia, Bartonella, Ehrlichia,* hemotropic *Mycoplasma* and *Rickettsia* spp. DNA was not PCR amplified.

The 16S rDNA sequences from the agar subculture isolate and mitral valve tissue were identical to each other and shared 100% (581/581 base pairs) homology with *Pasteurella canis* (GenBank accession No. JN713438.1).

3.3. Matrix-assisted laser desorption/ionization – time of flight mass spectrometry

Using MALDI-TOF MS, the isolate was confirmed as *Pasteurella canis* with 99.9% confidence.

4. Discussion

To our knowledge, this report describes the clinical presentation, diagnostic evaluation, and microbiological and molecular characterization of the first recorded case of *Pasteurella canis* infective endocarditis in a dog. Among the reports of infections caused by the *Pasteurella* genus, *P. canis* accounted for 22% of cultures at a veterinary medical teaching hospital (UC Davis) in a four year period (Biberstein et al., 1991) and 50% of *Pasteurella* isolates from dog bite wounds in people (Talan et al., 1999). In the human medical literature, *P. canis* has been recognized increasingly as a zoonotic agent capable of causing numerous types of infections besides superficial infections as well, including lower respiratory tract infections (Bhat et al., 2015), osteomyelitis (Hara et al., 2002), septic arthritis (Hazelton et al., 2013), and systemic bacteremia (Albert and Stevens, 2010). Despite this variety of infection types across host species, except for a brief report of systemic *P. canis* in dogs (Elsinghorst, 2003), the authors are unaware of any reports describing *P. canis* infective endocarditis in any animal species.

Infective endocarditis is an illness affecting between 0.09% and 6.6% of dogs, in which a microorganism, most commonly bacteria, infects the endocardium through a disruption in the endothelium (MacDonald, 2014). Endocardial disruption can be the result of mechanical (e.g. myxomatous mitral valve disease) or potentially immunological (e.g. systemic lupus erythematosus) injury (Calvert, 1982). As the vegetative lesion grows, its inner strata become protected from antibacterial agents and immune surveillance making antimicrobial elimination difficult to achieve. Bacteria can localize to the heart valve from various sources including urinary tract infection, pyoderma, wounds, or potentially periodontal disease (Peddle and Sleeper, 2007; Peddle et al., 2009). The most frequently reported organisms isolated from dogs with infective endocarditis include *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Bartonella* spp., and others less commonly including *Pseudomonas* spp., *Erysipelothrix rhusiopathiae*, *Enterobacter* spp., *Pasteurella multocida*, *Corynebacterium* spp., *Proteus* spp., and rare infections by *Actinomyces* spp. and an *Actinomyces canis*-like species (MacDonald, 2010; Balakrishnan et al., 2016). It is possible that the oral ulcer was the source of the dog's *P. canis* bacteremia with heart valve localization resulting in endocarditis.

In contrast to *P. canis*, *Pasteurella multocida* endocarditis has been described in dogs, and has been infrequently reported in immunocompetent and immunocompromised humans (Hombal and Dincsoy, 1992; Khan et al., 2012; Mikaberidz et al., 2013; Guilbart et al., 2015). Animal bite wounds are the most common source of *P. multocida* infective endocarditis, though reports of other routes of exposure including dogs licking open wounds and respiratory inoculation have been reported (Fukumoto et al., 2002; Albert and Stevens, 2010; Guilbart et al., 2015). A man with *P. multocida* septicemia without animal exposure suggests there are also other reservoirs for infection (Yuji et al., 2015). The dog in the present report had varied exposure through hunting activities, outdoor kenneling, unsupervised interactions with other dogs, and tongue ulceration—all considered putative sources for *P. canis* exposure.

Antemortem diagnosis of infective endocarditis is based on a combination of non-specific clinical signs, auscultation of a heart murmur, echocardiography and identification of the bacteria through blood cultures, although a large proportion (ranging 61 to 70%) of confirmed cases demonstrate no growth on routine bacteriological culture (Sisson and Thomas, 1984; MacDonald et al., 2004). Furthermore, intracellular pathogens such as *Bartonella* spp. are highly fastidious and require special culture techniques (Sykes et al., 2006). The dog in this report was diagnosed with infective endocarditis based on clinical signs (new heart murmur, fever, historical arthropathy, presumed vasculitis) and echocardiographic lesions (sessile hyperechoic nodule attached to the mitral valve), but was euthanized prior to the availability of blood culture results. Diagnosis was confirmed on post-mortem histopathological visualization of Gram-negative coccobacilli embedded within the vegetative lesion adhered to the mitral valve, by subsequent 16S rDNA PCR of *P. canis* from the heart valve, and isolate (100% sequence homology). The isolate was also confirmed as *P. canis* by MALDI-TOF MS.

In veterinary diagnostic laboratories, identification of *Pasteurellaceae* bacteria is done mainly with phenotypic assays, while genetic identification based on housekeeping genes is primarily used for research investigation of particularly important diagnostic specimens. As demonstrated in this study, 16S rDNA PCR and MALDI-TOF MS may represent promising alternatives to the currently practiced phenotypic diagnostics typically carried out in veterinary diagnostic laboratories (Cai et al., 2014).

Infective endocarditis carries a poor to grave prognosis, with 33% of dogs dying within the first week after diagnosis, and a reported 92% of dogs dying within 5 months, although those with mitral valve vegetative lesions have longer median survival times than those with aortic valve lesions (Sisson and Thomas, 1984; MacDonald et al., 2004). Prognosis can vary based on the location of the lesion, the infective agent, earlier diagnostic and therapeutic interventions, and the presence or absence of congestive heart failure at the time of infective endocarditis diagnosis. Death typically occurs secondary to congestive heart failure, thromboembolic disease, or elective euthanasia.

Potential shortcomings of this study include the inability to rule out coinfections as contributing factors in development of endocarditis, an unclear understanding of the bacterial source of infection, and the unfortunate inability to attempt treatment after rendering a diagnosis of infective endocarditis. Because this is an isolated single case, caution should be considered in extrapolation of these findings to other cases of infective endocarditis.

5. Conclusion

This study demonstrates an additional *Pasteurella* species that is capable of causing infective endocarditis in dogs. Increased vigilance and earlier diagnostic consideration of this difficult-to-detect cardiovascular disorder may improve outcomes associated with medical treatment of infective endocarditis in dogs.

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Conflict of interest declaration

In conjunction with Dr. Sushama Sontakke and North Carolina State University, Edward B. Breitschwerdt, DVM holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is a co-founder, shareholder and Chief Scientific Officer for Galaxy Diagnostics, a company that provides advanced diagnostic testing for the detection of *Bartonella* spp. infections. The remaining authors have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2018.12.001.

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