Erysipelothrix rhusiopathiea Infectious Endocarditis and Putative Secondary Immune Mediated Hemolytic Anemia and Thrombocytopenia in a Dog, and a Literature Review

Sugar, N.,*[1] Paitan, Y.,[2] Merhavi, N.,[1] Aroch, I.[1] and Kelmer, E.[1]

[1] Departments of Small Animal Emergency and Critical Care (N. Sugar, E. Kelmer), Diagnostic Imaging (N. Merhavi) and Small Animal Internal Medicine (I. Aroch), The Hebrew University Koret School of Veterinary Medicine-Veterinary Teaching Hospital (KSMV-VTH), P. O. Box 12, Rehovot, 761001, Israel.

[2] Clinical Microbiology Laboratory, Meir Medical Center, Tchernichowski 59, Kfar Saba, 44282, Israel and Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, 39978, Israel.

* **Corresponding author:** Dr. Noam Sugar, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem P.O. Box 12, Rehovot, 761001, Israel
Email: noam.sugar@mail.huji.ac.il

ABSTRACT

This report describes a case of a 7-year spayed female Vizsla dog, presented to the The Hebrew University Koret School of Veterinary Medicine (HUVTH) and diagnosed with infectious endocarditis due to *Erysipelothrix rhusiopathiae* infection based on echocardiology, blood culture and putative secondary immune mediated hemolytic anemia and thrombocytopenia (Evans syndrome), and secondary left iliac artery thromboembolism. The putative exposure route to the bacterium, the prognosis and secondary complications are discussed, in light of a literature review.

Keywords: Canine; Septic Embolism; Echocardiography; Hemolysis; Evans syndrome; Bacteremia; Blood culture.

INTRODUCTION

Infectious endocarditis (IE) is an uncommon life-threatening disorder in dogs, caused by endocardial colonization by microorganisms (1, 2), mostly bacteria, although rickettsiae and fungi may be causative agents (3). The causative bacteria most often reported in dogs with IE include *Streptococcus* spp., *Staphylococcus* spp., and *Escherichia coli* (4, 5), and less commonly include species of *Corynebacterium*, *Pasteurella* and *Bartonella*, as well as *Pseudomonas aeruginosa* and *Erysipelothrix rhusiopathiae* (6-12).

Bacterial colonies form endocardial proliferative or erosive lesions, leading to valvular dysfunction, serving as sources for septic emboli and thromboembolism (1), affecting various body organs, and causing infractions and metastatic infection (3). The prevalence of IE in dogs, based on necropsy results, ranges from 0.09% to 6.6% (2), although its true prevalence is probably higher, due to under-diagnosis (2). Dogs of medium to large, mostly purebred breed, of middle-age and being male are risk factors for IE (2). German shepherd, Boxer, Golden and Labrador retriever and Rottweiler are overrepresented breeds in cohorts of IE in dogs (3).

Transient or persistent bacteremia is a perquisite for IE (2). The initiating bacteremic event is often unapparent, although occasionally, IE may be linked to former illnesses, which might have induced bacteremia (3). IE might result from primary skin, oral cavity, urinary tract, prostate, lung or other organ infections and secondary to invasive medical procedures (e.g., endoscopy, urethral catheterization and anal surgery) (4). The prevalence of bacteremia-associated dental procedures in dogs is as high as 85%, however, only a minority of such cases eventually develop IE (4).

Development of IE depends on several factors, including presence of endothelial damage and blood flow disturbances, the host's immunity and the causative microorganism's virulence (1). Typically, the aortic valve (AV) and mitral valve (MV) are involved, due to the left heart's higher blood pressure and velocity, subjecting them to constant micro-trauma with their motion (3). Dogs with congenital heart disease mostly have low prevalence of IE, excluding subaortic stenosis and patent ductus arteriosus, where volume overload and abnormal jet flow induce endocardial lesions (13, 14). Conditions inducing impaired immunity or hypercoagulability, such as malignancy, immune mediated diseases, diabetes mellitus, hyperadrenocorticism and immunosuppressive therapy (e.g., glucocorticoids) may predispose to IE (1, 3).

The history and clinical signs of dogs with IE are non-specific, varying depending on the particular primary organ involvement. Lameness is the most frequently recognized clinical sign, followed by lethargy, anorexia, respiratory abnormalities, weakness, recurrent fever, weight loss and abdominal pain (1, 2), and possibly, signs secondary to immune complex disease, immune mediated disease and congestive heart failure (CHF) (3). The presence of heart murmur, especially if previously undocumented, raises suspicion of IE.

The presumptive diagnosis of IE relies on complete blood count (CBC), urinalysis, echocardiography and blood culture, while a definitive diagnosis requires pathological examination of the affected valve, which is clinically non-feasible *in vivo* (2). A scoring system, adapted from the modified Duke criteria for IE in humans can be used to determine the likelihood of IE, and includes major and minor criteria (2). The major criteria include echocardiographic evidence of vegetative or erosive lesions and newly recognized AV insufficiency, and two positive blood bacterial or fungal cultures (three, if the isolated microorganism is a common skin contaminant). The minor criteria include presence of fever, being a dog of medium to large breed, a concurrent thromboembolic disease, a concurrent immune mediated disease, a positive blood culture, unfulfilling the above-mentioned, corresponding major criterion, and the unofficially accepted criterion of *Bartonella* serological titter ≥1:1024 (2).

This report describes a case of a 7-year Vizsla dog diagnosed with *E. rhusioathaiea* IE and concurrent, putative secondary Evans syndrome and left iliac arterial thromboembolism (ATE).

CASE REPORT

A 7-year-old spayed female Vizsla dog was referred to the The Hebrew University Koret School of Veterinary Medicine due to fever of unknown origin (FUO), abnormal blood test results and paraparesis. It was currently vaccinated, dewormed and treated prophylactically every three months against *Spirocerca lupi*. The dog lived indoors, with access to a closed yard in Gedera, Israel, and fed a commercial diet. Ten weeks before presentation, it underwent surgical excision of cutaneous masses in the eyelid and anal area, both histopathologically diagnosed as melanoma. The pre-operative CBC was unremarkable. Six weeks later, the dog showed weakness, anorexia and episodic left hind limb lameness. Physical examination at the referring clinic showed fever (39.8° C), bilateral conjunctivitis with purulent ocular discharge and muscular pain caudal to the left knee. CBC showed marked leucocytosis and thrombocytopenia (Table 1). Stained blood smear microscopic examination confirmed the automated platelet count, and showed neutrophilic leucocytosis with marked left shift. Serum chemistry was unremarkable (Table 2). The dog was tentatively diagnosed with monocytic ehrlichiosis, and prescribed doxycycline (5 mg/kg PO q12h for 10 days; brand unknown), amoxicillin-clavulanic acid (25 mg/kg PO q12h for 10 days, brand unknown) and firocoxib (11.4 mg/kg, PO q24h for 7 days, brand unknown), and showed some clinical improvement on the following day.

Two weeks later, the dog showed recurrent signs of weakness, was represented to the referring clinic, and showed bilateral conjunctivitis with purulent ocular discharge and fever (39.8° C). CBC showed recurrent granulocytic leucocytosis with monocytosis (Table 1), severe thrombocytopenia, confirmed by microscopic blood smear examination, and moderate anemia. Immune-mediated thrombocytopenia (IMT) was suspected. Prednisone (1 mg/kg PO q24h for 7 days; brand unknown), azathioprine (1.25 mg/kg PO q24h for 14 days; brand unknown) and doxycycline (5 mg/kg PO q12h for 21 days; brand unknown) were prescribed. The dog had improved over the next two days, but deteriorated one day later. Repeat CBC showed neutrophilic leucocytosis and thrombocytopenia (Table 1). Microscopic blood smear examination confirmed the thrombocytopenia, and showed neutrophilia with left shift, 'reactive' monocytes and unspecified degree of polychromasia, anisocytosis and metarubricytosis. The above-mentioned treatment was continued for five

Table 1: Hematological and hemostatic test results of a 7-year old spayed female Viszla dog diagnosed with infectious endocarditis, secondary Evans syndrome and iliac artery thromboembolism.

Analyte	rDVM 22.12.16	rDVM 8.1.17	rDVM 11.1.17	rDVM RI	KSMV-VTH 17.1.17	KSMV-VTH RI
Leukocytes (10^3/μL)	40.8	34	41.3	6.0-17.0	NA	5.05-16.76
Erythrocytes (10^6/μL)	5.94	3.51	3.41	5.5-8.5	NA	5.65-8.87
Hematocrit (%)	42.9	25	25	37.0-55.0	NA	37.1-61.7
Platelets (10^3/μL)	75	22	37	200-500	37.5*	148-484
MCV (fL)	72	71	73	60.0-72.0	NA	61.6-73.5
MCHC (g/dL)	31.1	34.7	31.4	34.0-38.0	NA	32-37.9
RDW (%)	13.2	13.3	13.5	12.0-16.0	NA	13.6-21.7
MPV (fL)	10.6	10.5	11.3	6.1-10.1	NA	8.7-13.2
Lymphocytes (10^3/μL)	3	3	3.6	1.2-4.5	0*	1.05-5.1
Monocytes (10^3/μL)	1.8	1.6	2.5	0.3-1	28*	0.16-1.12
Granulocytes (10^3/μL)	36	29.4	35.2	3.5-12	NA	NA
Prothrombin time (sec)	NA	NA	NA		7.95	6-8.4
aPTT (sec)	NA	NA	NA		15	11-17.5
D- Dimer (ng/mL)	NA	NA	NA		8	<250
Antithrombin activity (%)	NA	NA	NA		90	87-140

rDVM, performed at the referring clinic; KSMV-VTH, performed at presentation to the Hebrew University Koret School of Veterinary Medicine- Veterinary Teaching Hospital; RI, reference interval; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; aPTT, activated partial thromboplastin time; *, manual platelet count made on stained blood smear.

additional days, but the dog failed to improve, and one day before presentation to the hospital, was unable to bare weight on its hind limbs for several hours.

At presentation to the hospital, the dog was quiet, alert and responsive, with heart rate of 140 bpm, respiratory rate of 36 breaths/minute and rectal temperature of 40.2˚C. The oral mucous membranes were pink. Mild bilateral ocular purulent discharge, abdominal skin petechiae and ecchymoses and a weak left femoral pulse were noted. Thoracic auscultation revealed left-sided, grade IV/VI systolic heart murmur and I/IV diastolic murmur at the heart base level, and grade III/VI systolic heart murmur at the heart apex level and right-sided, grade II/VI systolic heart murmur at the heart base level. Intermittent left hind limb lameness and proprioceptive deficits were noted, while the withdrawal and patellar reflexes were normal. The oscillometric (Cardell, Midmark, Tampa, FL, USA) systolic and diastolic arterial blood pressures were 161 mmHg and 93 mmHg, respectively.

Due to financial constraints, a full CBC was not performed at presentation. The packed cell volume (PCV) was low (26%; RI: 37-55). Stained (modified Wright's staining solution) blood smear examination revealed marked neutrophilic leukocytosis (segmented neutrophils, 63%; band neutrophils, 9%), monocytosis (28%) severe lymphopenia (lymphocytes, 0%) (Table 1), thrombocytopenia (manual platelet count, 30-45x10^3/μL), giant platelets, mild neutrophil cytoplasmic toxicity, markedly reactive monocytes, mild anisocytosis, polychromasia, macrocytosis and metarubricytosis, Howell-Jolly bodies and moderate spherocytosis (60% of the RBCs). Osmotic RBC fragility testing was positive. In-saline slide agglutination test at room temperature was negative. Serum chemistry (Cobas 6000, Roche, Mannheim, Germany; at 37˚ C) showed mild to moderately increased activities of hepatobiliary enzymes (Table 2).

Urinalysis showed specific gravity of 1.020, moderate proteinuria (150 mg/dL) and mild leucocyturia and bacteriuria. Urine culture was declined due to financial constraints. The prothrombin time (PT), activated partial thromboplastin time (aPTT) (Thrombostat Benhnk Elektoronic; Norderstedt, Germany), D-dimer concentration (Cobas 6000; Roche, Mannheim, Germany) and antithrombin activity (ACL 9000; Instrumentation Laboratory, Milano, Italy) were within their reference intervals (Table 1).

Abdominal ultrasonography (Mindray, DC-8, microconvex probe, 3-11 MHz) scan demonstrated mild renal heterogeneous echogenicity and reduced renal corticomedullary differentiation. In the caudal pole of the left kidney, a cyst-like structure, containing anechoic substance was noted. A 7-mm in diameter homogenous intra-aortic structure at the aortic bifurcation was noted, and color Doppler imaging showed that it partially occluded the aortic blood flow to the hind limbs (Figure 1). Echocardiography demonstrated thickening of the mitral valve leaflets, with vegetative lesions, that protruded into the left atrium. Vegetative lesions were also viewed on the AV leaflets (Figures 2A and 2B). A complete evaluation by a board-certified cardiologist was declined due to financial constraints.

Table 2: - Serum chemistry results of a 7-year old spayed female Viszla dog diagnosed with infectious endocarditis, and secondary Evans syndrome and iliac artery thromboembolism.

Analyte	rDVM 22.12.16	rDVM RI	KSMV-VTH 17.1	KSMV-VTH RI
Total Bilirubin (mg/dL)	0.3	0-0.9	0.13	0.0-0.2
Cholesterol (mg/dL)	230	110-320	250.3	135-361
Total CO2 (mmol/L)	NA	NA	17.6	16-26
Creatinine (mg/dL)	0.5	0.5-1.8	0.58	0.3-1.2
Glucose (mg/dL)	81	70-143	88.9	64-123
Triglycerides (mg/dL)	NA	NA	93.8	19-133
Urea (mg/dL)	14.98	14.98-57.78	10.6	10.7-53.5
Albumin (g/dL)	2.4	2.2-3.9	3.04	3.0-4.4
Globulin (g/dL)	3.6	2.5-4.5	2.88	2.5-4.5
Total protein (g/dL)	6	5.2-8.2	5.92	5.2-8.2
Alanine transaminase (U/L)	23	10-100	151.7	19-67
Alkaline phosphatase (U/L)	152	23-212	951	21-170
Amylase (U/L)	556	500-1500	888	103-1510
Aspartate transaminase (U/L)	NA	NA	54.8	19-
Creatine kinase (U/L)	NA	NA	168	51-399
γ-glutamyl transferase (U/L)	6	0-7	44	0-6
Lipase* (U/L)	522	1800	NA	
Calcium (mg/dL)	8.9	7.9-12	8.36	9.7-11.5
Chloride (mmol/L)	NA	NA	108.3	108-118
Phosphorus (mg/dL)	4.8	2.5-6.8	3.81	3-6.2
Potassium (mmol/L)	NA	NA	3.83	3.6-5.3
Sodium (mmol/L)	NA	NA	146	145-154

rDVM, measured at the referring clinic; KSMV-VTH, measured at presentation to the Hebrew University Koret School of Veterinary Medicine- Veterinary Teaching Hospital; RI, reference interval; NA, not available; *, total serum lipases.

Blood samples for bacterial culture were obtained under proper aseptic technique, and injected into aerobic and anaerobic blood culture bottles (BACTEC™ Plus Aerobic/F and BACTEC™ Plus Anaerobic/F Culture; Becton Dickinson, Sparks, NV, USA) and incubated (BACTEC™ FX unit; Becton Dickinson, Sparks, NV, USA). Positive growth was detected on the next day, flagged positive by the BACTEC FX unit. Direct microscopic Gram staining showed presence of Gram-positive rods. Samples were cultured on 5% sheep blood, Columbia CNA, CDC anaerobic blood (HyLab, Rehovot, Israel), chocolate, MacConkey and CDC anaerobic Blood+gentamycin (NOVAmed, Jerusalem, Israel) plates. Growth was observed on the next day. Bacterial colonies on the blood agar plate were small, transparent and catalase negative, with a narrow alpha hemolysis zone. Bacterial identification was performed directly from the colonies by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (VITEK MS, BioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing was performed both directly on the positive blood culture, and on the next day, from colony suspension as well, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (15).

Based on the history, clinical signs and the laboratory and imaging findings, before bacterial culture results became available, the dog was tentatively diagnosed with IE. The transient left hind limb lameness, paraparesis and proprioceptive deficits were attributed to temporary, partial arterial blood flow obstruction to the hind limbs by a thrombus or embolus, as demonstrated by Doppler ultrasonography. The moderate regenerative anemia and severe thrombocytopenia were likely due to immune mediated hemolytic anemia (IMHA; based on the spherocytosis and positive osmotic fragility) and thrombocytopenia (IMT), respectively (i.e., Evans syndrome), secondary to the systemic infection and inflammation. Inflammation might have also contributed to the anemia (i.e., anemia of chronic disease). Other complications, including the increased hepatobiliary enzyme activities and the urinary tract infection were attributed to the systemic infection and inflammation. The left kidney's structural changes and the renal cyst were likely incidental findings.

The dog was hospitalized. Treatment included IV fluids (lactated ringer solution, Teva Medical, Ashdod, Israel; at 0.46 mL/kg), azithromycin (Zythromax, Pfizer, New York City, NY, USA; 10 mg/kg IV q24h), enrofloxacin (Baytril, Bayer Animal Health, Leverkusen, Germany; 10 mg/kg slow IV q24h) and amoxicillin- clavulanic acid (Augmentin, GSK, Brentford, UK; 16.25 mg/kg IV q12h). As the left heart base diastolic heart murmur was indicative of AV insufficiency, posing a risk for CHF, pimobendan (Cardiosure, Eurovet, Bladel, Holland; 0.325 mg/kg PO q12h) and enalapril (Enaladex, Dexcel Pharma, Or Akiva, Israel; 0.5 mg/kg PO q12h) were added. Azathioprine and doxycycline were discontinued, while prednisone dosage was gradually tapered.

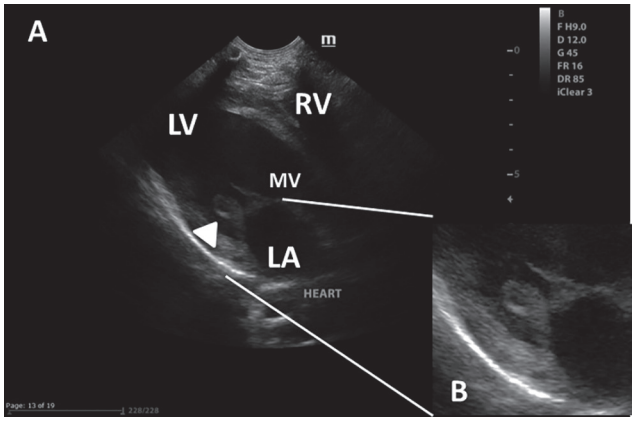

Figure 1: Ultrasonographic view of the left side of the heart of a 7-year old spayed female Viszla dog, diagnosed with infectious endocarditis, secondary Evans syndrome and iliac artery thromboembolism (A). A thickened, hyperechoic irregularity, deemed a vegetative lesion is present on the parietal mitral cusp (arrowhead). This vegitative lesion is enlarged in Figure 2. Abbreviations: Left atrium (LA); mitral valve (MV); left ventricle (LV); right ventricle (RV).

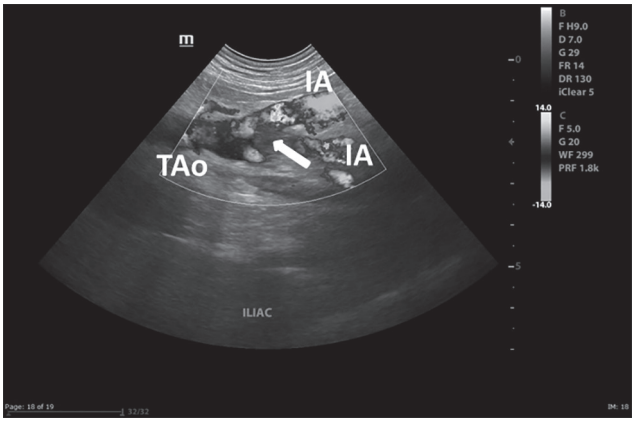

Figure 2: A sagittal ultrasonographic view of the trifurcation of the aorta and iliac arteries of a 7-year old spayed female Viszla dog diagnosed with infectious endocarditis, secondary Evans syndrome and iliac artery thromboembolism. A large thrombus or embolus is evident. Doppler image shows turbulent aortic blood flow surrounding the intraluminal filling defect (white arrow), with partial , almost complete blood flow obstruction to the iliac arteries. Abbreviations: Terminal aorta (TAo); iliac artery (AI).

The next day, melena and increased respiratory effort were noted. Right lateral and dorso-ventral thoracic radiographs demonstrated a diffuse interstitial pattern most noticeable in the caudo-dorsal lung area, which might have been compatible with non-cardiogenic pulmonary edema, pulmonary hemorrhage or pneumonia. The PCV was decreased (19%; RI: 37-55%).

At this point, against medical advice, the owners elected to discharge the dog and continue treatment and monitoring at the referring clinic. The owner was advised to continue IV antibiotics for 10 days at least; after which, their oral preparations were to be administered for three months. Pimobendan and enalapril were also prescribed. Prednisone was to be tapered cautiously, while closely monitoring the CBC for anemia and thrombocytopenia. Finally, anticoagulant treatment was to be considered later, once the platelet count will have been normalized, to prevent further thromboembolism. One day post-discharge, the dog was euthanized at the referring clinic, at the owners' request, due to deterioration of the anemia and financial and emotional constraints. Necropsy was declined. One day later, the bacterial blood culture and antibiotic susceptibility results became available. Culture yielded pure growth (99.9%) of *E. rhusiopathaiea*, sensitive to ampicillin, amoxicillin-clavulanic acid, penicillin G, ceftriaxone, cefuroxime, clindamycin and erythromycin, and was vancomycin-resistant.

DISCUSSION

The present dog showed many clinical signs and laboratory abnormalities typical of IE, and was at risk for IE due to the immunosuppressive prednisone and azathioprine treatment. Based on the modified Duke scoring system, it fulfilled two major criteria of IE (i.e., AV insufficiency indicated by the heart-base diastolic murmur and positive echocardiogram) and five minor criteria (i.e., fever, being a medium to large, pure-bred dog, one postive blood culture and presence of immune-mediated and thromboembolic diseases) (2). The definitive diagnosis of IE requires valvular histopathology, as it is impossible to distinguish IE from valvular thickening due to degenerative valve disease (DVD) by echocardiography (5), and as AV DVD can occurs in medium-sized dogs (16). Since *in vivo* valvular sampling for histopathology and bacterial culturing is not clinically feasible, IE was presumptively diagnosed based on the modified Duke scoring system (2).

The overall prognosis for IE in dogs is poor, with mortality rates of 60% to 95% (17), and presently, several negative prognostic indicators were noted, including increased risk of CHF, occurrence of Evans syndrome, iliac aortic thromboembolism (ATE) with possible bacterial embolism, urinary tract infection and hepato-billiary injury, concurrent immunosuppressive therapy, and finally, lack of owner's compliance due to financial constraints (2, 3, 7, 17).

This dog was at high risk for developing CHF, with both aortic and mitral valve insufficiency, contributing to increased left ventricular end-diastolic volume and pressure, which might have ultimately led to pulmonary congestion and edema (18). Dogs with IE and aortic insufficiency develop CHF more rapidly than those with mitral insufficiency, and have a significantly shorter median survival time (45 days vs. 330 days, respectively) (8). In a previous study, 5/10 dogs with AV IE were positive for *Bartonella* spp., and their prognosis was less favorable compared to other IE types (8), suggesting that the direct effect of aortic valve IE on survival cannot be separately evaluated from the causative organism effect. In another study, the prognosis of dogs with non-*Bartonella* spp.-related AV IE was better. Only a third of the dogs died or were euthanized within the first week, while 87% of the remaining ones survived for ≥6 months (19). Although the present dog was at high risk for developing CHF, the pulmonary edema noted upon thoracic radiography was assessed as non-cardiogenic, while pulmonary venous congestion, suggestive of CHF (20), was absent.

This dog showed progressive anemia and thrombocytopenia, and their pathogenesis remains putative. In 50-60% of dogs with IE, mild normocytic-normochromic non-regenerative anemia associated with chronic inflammation is noted (2), while mild to severe thrombocytopenia also occurs (18). In this dog, the marked spherocytosis and positive osmotic fragility test, with absence of Heinz bodies, were highly suggestive of immune mediated hemolysis (21, 22), although the in-saline slide agglutination test was negative. The reported sensitivity of the latter in dogs with IMHA ranges between 40% and 89% (23). Negative results occur when serum agglutinating autoantibodies titers are low (23). Therefore, a negative result cannot conclusively exclude IMHA. Coombs' testing was warranted, although it also suffers from false negative and false positive results (24, 25), but was declined due to the financial constraints. Other causes of anemia in this dog include thrombocytopenia-mediated bleeding, inflammation and mechanical RBC destruction, due to blood flow through the abnormal valves. Fragmentation hemolysis due to shear stress was proposed in several human patients with IE (26). Immune mediated diseases (e.g., polyarthritis and glomerulonephritis) are known consequences of IE, elicited by immune complex deposition (7), while IMHA and IMT occur sporadically in humans and dogs with IE (7, 26-28). Hemolytic anemia was reported in a 19-year-old man with *Streptococcus oralis* IE, diagnosed based on spherocytosis observed on blood smear examination and a positive direct Coombs' test (26). Evans syndrome was tentatively diagnosed based on presence of anemia, thrombocytopenia, increased unconjugated bilirubin and positive direct Coombs' test in a human infant with fungal IE (27). In the two reports of dogs with IE presumptively diagnosed with concurrent IMHA, the supportive diagnostic findings of IMHA were not described (7, 28). Bacterial infections have been associated with IMHA and IMT, but their role has not been completely established, however, molecular mimicry, autoantigen modulation and exposure of hidden antigens normally unexposed to the immune system, are potential mechanisms (29). Induction of IMT secondary to bacterial IE has more compelling evidence than induction of IMHA. IMT with positive for platelet anti-glycoprotein antibodies has been reported in humans with *S. aureus* and *Cardiobacterium hominis* IE (30). In dogs diagnosed with IE by the modified Duke's criteria, 1/18 cases was thrombocytopenic and anti-megakaryocyte antibody-positive (8). Another dog, with progressive neurological signs and thrombocytopenia, initially treated for IMT, was eventually diagnosed with *Staphylococcus* spp. IE and bacterial meningitis (31). Other mechanisms potentially contributing to thrombocytopenia in IE include consumptive coagulopathy (unlikely in this case, with the unremarkable hemostatic test results), consumption during clot formation, vasculitis and decreased bone marrow platelet production (7).

In addition, this dog had sustained ATE, visualized both on abdominal ultrasound and Doppler sonography. Thromboembolism is an important cause of morbidity and mortality in IE. In humans, the overall prevalence of thromboembolic events is 35%, ranging from 8% to 64%, and 20% of these are 'silent', detected only on CT scan (32), and most commonly occur in the cerebral arteries, spleen, kidneys, lungs and peripheral arteries (32, 33). The known risks factors for thromboembolism include valvular vegetation size >10 mm and severe mobility and mitral valve involvement (33), while early surgical intervention and antibiotic treatment decrease its likelihood (32). Use of platelets inhibitors and anticoagulants is controversial. Anticoagulant therapy has been shown to prevent thromboembolism in IE, but may increase the risk of intracerebral bleeding (34). In 44% of 71 dogs with IE, thromboembolic events were either suspected or confirmed by ultrasonography or necropsy (7). In another

study of IE in dogs, as many as 71% had thromboembolism, however, the diagnostic methods were unspecified in most cases (8).

Serious financial constraints, limited further diagnostics (e.g., full echocardiography, urine culture and several blood cultures over time) and therapy (e.g., longer in-hospital treatment, blood transfusion and advanced immunosuppressive therapy; e.g., other immunosuppressive drugs and human intravenous immunoglobulin therapy dosing). The initial treatment cost of IE reported in dogs was approximately 2000 USD, excluding subsequent recheck examinations due to complications, monitoring, and long-term treatment (7).

Nevertheless, several positive predictors of survival in this case are noteworthy, including absence of azotemia and CHF, and a relatively good overall clinical status, namely, the dog being alert and responsive, with relatively normal vital signs, good appetite and ability to ambulate. We therefore believe that given the chance to receive more intensive care, the outcome might have been successful.

The presently isolated bacterium, by blood culture, *E. rhusiopathiae*, is a small, Gram-positive, facultative anaerobe rod, with worldwide distribution (35, 36). It is most important in swine medicine and industry, causes swine erysipelas ("diamond-skin disease"), joint disease in sheep and cattle, and septicemia in turkeys, ducks and laboratory mice (35). It has zoonotic potential, mostly related to occupational exposure of workers in direct contact with infected animals and related products (e.g., farmers, fishermen, butchers, slaughterhouse workers and veterinarians), who are particularly at risk (29). Direct cutaneous contact is the common transmission route, although bacteremia occurs after undercooked pork consumption (37). Systemic *E. rhusiopathiae* infections unrelated to occupational exposure may occur, possibly due to oropharyngeal or gastrointestinal colonization (37). Infected dogs do not seem to pose public health hazards; although few reports of animal bites of humans suggested that *E. rhusiopathiae* may rarely be part of the oral flora of cats and dogs. *E. rhusiopathiae* infection in humans may manifest in three forms, including local cutaneous ("erysipeloid") form, generalized cutaneous form and systemic infection, which occurs rarely as a sequel of local infection (37). Over 90 cases of bacteremia have been reported in humans, mostly IE, which tend to occur in immunocompromised patients (37).

In dogs, *Erysipelothrix* spp. infections cause arthritis and discospondylitis (36) and IE (2), and can cause IE with intravenous inoculation (9). Nevertheless, the exposure route and the infective strains in dogs both remain unclear. The seroprevalence of *E. rhusiopathiae* was 5% in 120 stray dogs, suggesting environmental exposure (38). Such exposure was suspected also in a dog with diamond shaped erythematous skin lesion, compatible with erysipeloid, where *E. rhusiopathiae* was isolated by blood culture (36), because four days before onset of clinical signs, the dog was observed chewing wild turkey carcass bones, a possible exposure source, however, the carcass remains were unavailable for culture, so this hypothesis was not confirmed (36). In the present case, the source of infection remains unknown, but might have been the surgical site at the eyelid or near the anus.

The genus *Erysipelothrix* includes two species, *E. rhusiopathiae* and the newly recognized *E. tonsillarum* (9), each including several serovars, with different strains. It is unclear which *Erysipelothrix* strains lead to IE in dogs. Strains of *E. tonsillarum* serovar 7 were isolated from seven dogs with IE (9, 39), however, the epidemiology this serovar is unclear, but it was isolated from healthy pigs, pig slurry, pork, fish (39) and chicken meat samples (40). *E. tonsillarum* (unknown serovar) was also isolated from pig and sheep carcasses in an abattoir in West Australia (41). It is unknown if these bacterial sources have any association with *Erysipelothrix* infection in dogs.

It noteworthy that the blood culture yielded pure *E. rhusiopathiae* growth despite the prior antibiotic therapy this dog had received. Only a single blood sample has been cultured in this case, which does not comply with the recommendation to obtain multiple consecutive blood samples, from different venipuncture sites for culture when IE is suspected, to increase sensitivity (16, 43). The use of resin-containing blood culture medium in this case, which effectively inhibits doxycycline's antimicrobial activity, possibly allowed bacterial growth in culture (44). Although the isolated bacterium was sensitive to amoxicillin-clavulanic acid, the short treatment course with this antibiotic done one month prior to presentation to the hospital likely did not eliminate infection, possibly due to decreased drug distribution into the valvular vegetations (3). The initial culture results became available as early as two days from submitting the samples, and could guide treatment then, while the full antibiotic susceptibility profile became available one day later, and had the dog been still alive, would have possibly lead to a more favorable outcome, by shortening the duration of empirical antibiotic therapy.

Nevertheless, these antibiotic culture and sensitivity results do support the empirical choice of antibiotics in this case, and provide additional therapeutic options.

REFERENCES

1. Ware, W.A.: Acquired valve diseases. In: Ware, W.A. (Ed.): Cardiovascular Diseases in Small Animal Medicine. Manson Publishing, London, pp. 272-277, 2011.
2. Ljungvall, I. and Häggström, J.: Adult-onset valvular heart disease. In: Ettinger S.J., Feldman E.C. and Cote E. (Eds.): Textbook of Veterinary Internal Medicine 8th edn. Saunders-Elsevier, Philadelphia, pp. 3057-3062, 2017.
3. Calvert, C.A. and Thomason, J.D.: Cardiovascular infections. In: Greene, C.E.: Infectious Diseases of the Dog and the Cat 4th edn. Saunders, Saint Lois, pp. 925-933, 2012.
4. Calvert, C.A., Greene, C.E. and Hardie, E.M.: Cardiovascular infections in dogs: epizootiology, clinical manifestations and prognosis. J Am Vet Med Assoc. 187:612-616, 1985.
5. Miller, M.W., Fox, P.R. and Saunders, A.B.: Pathologic and clinical features of infectious endocarditis. J Vet Cardiol. 6:35-43, 2004.
6. Wall, M., Calvert, C.A. and Greene, C.E: Infective endocarditis in dogs. Compend Contin Edu Pract Vet. 24:614-625, 2002.
7. Sykes, J.E., Kittleson, M.D., Chomel, B.B., MacDonald, K.A. and Pesavento, P.A.: Clinicopathologic findings and outcome in dogs with infective endocarditis: 71 cases (1992-2005). J Am Vet Med Assoc. 228:1735-1747, 2006.
8. MacDonald, K.A., Chomel, B.B., Kittleson, M.D., Kasten R.W., Thomas, W.P. and Pesavento, P.A.: A prospective study of canine infective endocarditis in Northern California (1991-2001): emergence of *Bartonella* as a prevalent etiologic agent. J Vet Intern Med. 18:56-64, 2004.
9. Takahashi, T., Tamura, Y., Yoshimura, H., Nagamine, N., Kijima, M. and Nakamure, M.: *Erysipelothrix tonsillarum* isolated from dogs with endocarditis in Belgium. Res Vet Sci. 54:264-265, 1993.
10. Anderson, C.A. and Dubielzig, R.R.: Vegetative endocarditis in dogs. J Am Anim Hosp Assoc. 20:149-152, 1984.
11. Breitschwerdt, E.B., Kordick, D.L., Malarkey, D.E., Keene, B., Hadfield, T.L. and Wilson, K.: Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J Clin Microbiol. 33:154-160, 1995.
12. Breitschwerdt, E.B.: *Bartonella* species as emerging vector transmitted pathogens. Comp Contin Edu Pract Vet. 25(Suppl):12-15, 2003.
13. Aoki, T., Sunahara, H., Sugimoto, K. Ito, T., Kanai. E. and Fujii, Y.: Infectious endocarditis of the aortic valve in a Border collie dog with patent ductus arteriosus. J Vet Med Sci. 77:331-336, 2015.
14. Kienle, R.D., Thomas, W.P. and Pion, P.D.: The natural clinical history of canine congenital subaortic stenosis. J Vet Intern Med. 8:423-431, 1994.
15. Hindler, J.A., Richter, S.S., Bernard, K., Bodeis- Jones, S., Castanheira, M., Citron, M.D., Couturier M.R., Fritsche, T.R., Humphries, R.M., Jorgensen, J.H., Killian, S.B., Kohner, P., Matuschek, E., McDermott, P. and Patel, S.: Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria 3rd edn.; Clinical and Laboratory Standards Institute, Wayne, M45: 28-29, 2015.
16. Ware, W.A.: Acquired valve Diseases, Ware W.A.: Cardiovascular Diseases in Small Animal Medicine. Manson Publishing, London, p. 264, 2011.
17. Costello, M.F: Endocarditis. In: Silverstein, D. and Hopper, K. (Eds.) Small Animal Critical Care Medicine. Saunders, Saint Louis. pp. 486-490, 2009.
18. Macdonald, K.: Infective endocarditis in dogs: diagnosis and therapy. Vet Clin North Am Small Anim Pract. 40:665-684, 2010.
19. Sisson, D., Thomas, W.P.: Endocarditis of the aortic valve in the dog. J. Am. Vet. Med. Assoc. 184:570-577, 1984.
20. Ware, W.A.: Overview of cardiac radiography. Ware W.A.: Cardiovascular Diseases in Small Animal Medicine. Manson Publishing, London, pp. 43, 2011.
21. Piek, C.: Immune-mediated hemolytic anemias and other regenerative anemias. In: Ettinger, S.J., Feldman, E.C. and Cote, E. (Eds.): Textbook of Veterinary Internal Medicine 8th edn. Saunders-Elsevier, Philadelphia, pp. 2088, 2017.
22. Brockus, C.W.: Erythrocytes. In: Latimer K.S. (Ed.): Duncan and Prasse's Veterinary Laboratory Medicine, Clinical Pathology 5th edn; Wiley-Blackwell, Aims, pp. 20, 2011.
23. Balch, A., Mackin, A.: Canine immune-mediated haemolytic anemia: pathophysiology, clinical signs' and diagnosis. Compend. Cont. Educ. Pract. Vet. 29:217- 214, 2007.
24. Wardrop, K.J.: The Coombs' test in veterinary medicine: past, present, future. Vet. Clin. Pathol. 34:325-34, 2005.
25. Wardrop, K.J.: Coombs' testing and its diagnostic significance in dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 42:43-51, 2012.
26. Huang, H.L., Lin, F.C., Hung, K.C., Wang, P.N. and Wu, D.: Hemolytic anemia in native valve infective endocarditis – a case report and literature review. Jpn. Circ. J. 63:400-403, 1996.
27. Hsu, C.M., Lee, P.I, Chen, J.M., Huang, L.M., Wu, M.H., Chiu, I.S. and Lee, C.Y.: Fatal *Fusarium* endocarditis complicated by hemolytic anemia and thrombocytopenia in an infant. Pediatr Infect. Dis J. 13:1146-1147, 1994.
28. Jackson, M.L. and Kruth, S.A.: Immune-Mediated hemolytic anemia and thrombocytopenia in the dog: A retrospective study of 55 cases diagnosed from 1969 through 1983 at the Western College of Veterinary Medicine. Can. Vet. J. 26:245-250, 1985.
29. Karlsen, A.E. and Dyberg, T.: Molecular mimicry between nonself, modified self and self in autoimmunity. Semin. Immunol. 10:24-34, 1998.
30. Arnold, D.M., Smaill, F., Warkentin, T.E., Christjanson, L. and Walker, I.: *Cardiobacterium huminis* endocarditis associated with very severe thrombocytopenia and platelet autoantibodies. Am. J. Hematol. 76:737-377, 2004.
31. Bach, J.F., Mahony, O.M., Tidwell, A.S. and Rush, J.E.: Brain abscess and bacterial endocarditis in a Kerry Blue Terrier with a history of immune-mediated thrombocytopenia. J. Vet. Emerg. Crit. Care. 17:409-415, 2007.
32. Habib, G.: Embolic risk in subacute bacterial endocarditis: determinants and role of transesophageal echocardiography. Curr. Cardiol. Rep. 5:129-136, 2003.
33. Thuny, F., Disalvo, G., Belliard, O., Avierinos, J.F., Pergola, V.,

Rosenberg, V., Casalta, J.P., Gouvernet, J., Derumeaux, G., Iarussi, D., Ambrosi, P., Calabro, R., Riberi, A., Collart, F., Metras, D., Lepidi, H., Raoult, D., Harle, J.R., Weiller, P.J., Cohen, A. and Habib, G.: Risk of embolism and death in infective endocarditis: prognostic value of echocardiography a prospective multicenter study. Circulation. 112:69-75, 2005.

34. Maylonakis, E. and Calderwood, S.B.: Infective endocarditis in adults. N. Engl. J. Med. 345: 1318-1330, 2001.
35. Greene, C.E. and Prescott, J.F.: Gram-positive bacterial infections. In: Greene, C.E. (Ed.): Infectious Disease of the Dog and the Cat 4th edn. Saunders, Saint Lois, pp. 336-338, 2012.
36. Foster, J.D., Hartmann, F.A. and Moriello, K.A.: A case of apparent canine erysipeloid associated with *Erysipelothrix* bacteraemia. Vet. Dermatol. 23:528-530, 2012.
37. Reboli, M.C.: *Erysipelothrix rhusiopathiae*, 2010. Antimicrobe.org, E-Sun Technologies, Inc. Available at: http://www.antimicrobe.org/new/b76.asp; accessed at: 14.08.17.
38. Shimazaki, Y., Gamoh, K., Imasa, Y., Makie, H., Kanzaki, M. and Takahashi, T.: Detection of antibodies to *Erysipelothrix* in stray dogs in Japan. Acta Vet Scand. 46:159-161, 2005.
39. Eriksen, E., Fossum, K., Gamlem, H., Grondalen, J., Kucsera, G. and Ulstein, T.: Endocarditis in two dogs caused by *Erysipelothrix rhusiopathiae*. J. Small Anim. Pract. 28:117-123, 1987.
40. Nakazawa, H., Hayashidani, H., Higashi, J., Kaneko, K.I., Takahashi, T. and Ogawa, M.: Occurrence of *Erysipelothrix* spp. in chicken meat parts from a processing plant. J. Food Prot. 61:1207-1209, 1998.
41. Wang, Q., Fidalgo, S., Chang, B.J., Mee, B.J. and Riley, T.V.: The detection and recovery of *Erysipelothrix* spp. in meat and abattoir samples in Western Australia. J. Appl. Microbiol. 92:844-850, 2002.
42. Calvert, C.A. and Thomason J.D.: Cardiovascular infections, Greene C.E.: Infectious disease of the dog and The cat 4th ed, Saunders Co., Missouri, pp. 919, 2012.
43. Pfeltz, R.F. and Warns, M.T.: Blood culture antimicrobial removal effectiveness of BD Bactec Plus media versus BacT/ALRET FAN media. ASM Microbe, Washington DC, 2003. Available at: http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=3491A92D94EDBA304FBC0DA2B216B432?doi=10.1.1.370.6659&rep=rep1&type=pdf