Treatment of a cat with presumed *Bartonella henselae*-associated immune-mediated hemolytic anemia, fever, and lymphadenitis

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Abstract
A 2.5-year-old castrated male cat presented with fever and marked generalized lymphadenopathy of 4-months duration, despite treatment with amoxicillin-clavulanate/marbofloxacin. Abnormalities were not detected on complete blood count, serum chemistry, and FIV/FeLV test apart from a borderline, non-regenerative anemia. Peripheral lymph node fine needle aspirations revealed a marked increase in the percentage of intermediate- and lymphoblastic-lymphocytes in addition to reactive macrophages. Three weeks after presentation, the cat developed a severe, regenerative, immune-mediated hemolytic anemia (IMHA) which responded to immunosuppressive therapy. Fever and lymphadenopathy persisted. Peripheral lymph nodes tested positive for *Bartonella henselae* DNA in real-time PCR assay and sequencing. Treatment with pradofloxacin and doxycycline resulted in resolution of clinical signs, and negative PCR tests. Despite its reported low pathogenicity, *B. henselae* infection should also be considered in cats with protracted unexplained fever, lymphadenitis, and IMHA. Furthermore, a combination of pradofloxacin and doxycycline might be considered in cats with bartonellosis given its apparent clinical efficacy.

KEYWORDS
bartonellosis, feline, fever, lymphadenitis, lymphadenomegaly, pradofloxacin

1 | INTRODUCTION

A 2.5-year-old, strictly indoors, castrated male, British shorthair cat presented with a chief complaint of lethargy and inappetence of 2-day duration. Abnormal physical examination findings included a rectal temperature of 40.5°C, generalized lymphadenomegaly (including bilateral enlargement of the mandibular, prescapular and popliteal lymph nodes), a parasternal, systolic, crescendo heart murmur with occasional gallop rhythm, dehydration, mild peri-ocular and cervical seborrhea, and flea infestation. No abnormalities were initially noted in the CBC or serum chemistry analysis except for borderline anemia as judged by a PCV of 28% (normal reference interval [RI] of 30%-45%; Figure 1). No signs of regeneration were noted on a new-methylene blue-stained blood smear, and there were no red blood cell

Abbreviations: IMHA, immune-mediated hemolytic anemia; TS, total solids.

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morphology abnormalities. Abnormal sonographic findings included mild cystic duct dilatation and enlarged, hypoechoic cecal lymph-nodes. Abnormalities were not detected on chest x-rays. In the ensuing 2 weeks, the cat had thrice been hospitalized for 24-hours each time owing to recrudescence of fever and inappetence and was treated with Ringer’s lactate solution (IV), amoxicillin-clavulanate (Clavenir, Laboratorio Reig Jofre, Toledo, Spain; 15 mg/kg, IV, q12h), metamizole (Calmagine, Vetoquinol, Lure, France; 25 mg/kg, SC, q12h), mirtazapine (Medi-market pharmaceuticals, Emek Hefer, Israel; 3.75 mg/cat, topically, q24h) and after its second hospitalization, after anemia had worsened (PCV/total solids [TS] 19%/6 g/dL; Figure 1), also with marbofloxacin (Marbocyl, Vetoquinol, Lire, France; 4 mg/kg, PO, q24h). At home, the same medications were administered, with amoxicillin-clavulanate, PO, (Medi-market pharmaceuticals, Emek Hefer, Israel). Fipronil 0.25% spray (Frontline, Merial, Georgia) was topically applied for the flea infestation, with apparent resolution in the ensuing months.

After 3 weeks of treatment, with persistence of clinical signs, the cat’s PCV continued to decline, reaching a PCV/TS of 11%/6.5 g/dL, with a normocytic, normochromic anemia in its CBC, and a strong regenerative response upon examination of a new-methylene blue-stained blood smear with 1.5% to 2% aggregate reticulocytes and nucleated red blood cells. Marked agglutination was observed on 2 consecutive days in a saline agglutination test, which had been performed by mixing 1 drop of EDTA-anticoagulated blood with 4 drops of saline, at room temperature, followed by mixing and microscopic examination. Consequently, the cat was transfused with 2, A-type-matched packed red cells units (5-6 mL/kg, each unit, during the course of 2 days) and treatment with prednisolone was initiated (Medi-market pharmaceuticals, Emek Hefer, Israel; 2 mg/kg, PO, q12h) in addition to administration of mycophenolate mofetil (Vetmarket, Shoham, Israel; 11 mg/kg, syrup, PO, q12h). Additional diagnostic tests included an FIV/FeLV blood test (Fastest, Megacor Diagnostik GmbH, Gemeinde Hörbranz, Austria) which was negative.
for the presence of FIV antibody/FeLV antigen, respectively, and a
cytologic evaluation of mandibular and popliteal lymph nodes which
revealed an increase in the percentage of lymphoblasts, plasma cells
and the presence of highly reactive macrophages, some of which con-
tained phagocytosed cells (Figure 2). After the cat’s PCV/TS had stabi-
lized at 16%/6.2 g/dL, it resumed eating and was discharged with
instructions to administer prednisolone, mycophenolate mofetil,
marbofloxacin, omeprazole (Vetmarket, Shoham, Israel; 1.6 mg/kg,
q24, q24h) and mirtazapine.

The following 3 months were marked by resolution of the anemia
and the agglutination, with a maximal PCV/TS of 32%/6.2 g/dL
(Figure 1B), and a gradual tapering of prednisolone treatment (at 20%-25%
decrements), while mycophenolate mofetil and omeprazole treat-
ment remained unchanged. During that time, the cat presented twice
for inappetence and fever ($T = 40\,\degree C$), with mild generalized lym-
phadenomegaly. Treatment remained unchanged, apart from the addi-
tion of metamizole and mirtazapine for several days each time the cat
presented with fever. At the end of this period, and while on a tapering
regimen of prednisolone (1 mg/kg, every other day) and mycophenolate
mofetil treatment, the cat presented with fever, vomiting, soft stools,
weight loss, inappetence, and considerable exacerbation of the general-
ized lymphadenomegaly including the inguinal and mesenteric lymph
nodes. Fine needle aspiration of several lymph-nodes demonstrated the
same cytological picture as previously described. Owing to persistence
of lymphadenomegaly and fever, while the hemolytic anemia was in remission.

After the identification of $B.\, henselae$ DNA, a dual antibiotic treat-
ment comprising pradofloxacin (Veraflox, Bayer Animal Health GmbH,
Leverkusen, Germany; 4.5 mg/kg, PO, q24h, for 62 days) and doxycy-
cline (Doxylin, Dexcel Pharma, Or-Akiva, Israel; 11 mg/kg, PO, q24 h,
for 62 days) was prescribed. Three days after initiation of treatment
the cat underwent echocardiography and was diagnosed with hyper-
trophic cardiomyopathy and a dynamic left ventricular outflow tract
obstruction. There was a negligible amount of pericardial effusion,
with no signs of vegetative valvular lesions. Subsequently, enalapril
(Enaladex 5, Dexcel Pharma, Or-Akiva, Israel; 0.28 mg/kg, PO, q12h),
atenolol (Normalol 25, Dexcel Pharma, Or-Akiva, Israel; 1.4 mg/kg,
PO, q24h) and clopidogrel (Plavix, Sanofi, Amilly, France; 4.21 mg/kg,
PO, q24h) were added to the treatment. Generalized lym-
phadenomegaly was still present at the time.

**FIGURE 2** DiffQuick-stained smears of fine needle aspirations from prescapular and popliteal lymph-nodes of a cat with marked
lymphadenomegaly, hemolytic anemia and fever. $B.\, henselae$ DNA was later isolated from the lymph-nodes. (A) An increase in the
percentage of lymphoblasts and intermediate lymphocytes, which together constituted up to 40% to 50% of the lymphocyte population in some
of the fields, was documented; (B) Additionally, reactive, vacuolated macrophages, some of which phagocytosing suspected cellular material
(arrow), were infrequently observed

using the QIAamp DNA blood mini-kit (QIAGEN, Valencia, California).
The DNA sample was submitted to a HRM real-time PCR assay
targeting a fragment of approximately 200 bp of the 16S-23S internal
transcribed spacer (ITS), as previously described. Additional HRM
real-time PCR assay targeted the mRNA $ssrA$ gene (approx. 350 bp), as
previously described. Positive PCR products were sequenced using
the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM
3100 Genetic Analyzer (Applied Biosystems, Foster City, California).
DNA sequences were evaluated with the ChromasPro software version
2. 1.1 (Technelysium Pty Ltd, Australia) and compared for similarity with
sequences available in GenBank, using a BLAST program. The sample
was positive for the ITS and the $ssrA$ targeted loci. The BLAST analysis
revealed that both sequences obtained showed high identity to
$B.\, henselae$ (95.40% [166/174 base pairs) for the ITS locus and
99.61 to 99.62% [259/260 base pairs] for the $ssrA$ gene; Table 1).

Pending the PCR results, treatment included mycophenolate
mofetil and mirtazapine, with persistence of lymphadenomegaly and
fever, while the hemolytic anemia was in remission.

After the identification of $B.\, henselae$ DNA, a dual antibiotic treat-
ment comprising pradofloxacin (Veraflox, Bayer Animal Health GmbH,
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phadenomegaly was still present at the time.
**TABLE 1** Basic local alignment search tool results of the sequences obtained from the amplification of the ITS and ssrA target genes

| Description                          | Scientific name      | Query length | Max score | Total score | Target gene | Query cover | E value    | %       | length (bp) | Accession     |
|--------------------------------------|----------------------|--------------|-----------|-------------|-------------|-------------|------------|---------|-------------|---------------|
| Bartonella henselae strain GDC08 tmRNA (ssrA) gene, partial sequence | Bartonella henselae | 262          | 473       | 473         | ssrA        | 99%         | 3.00E−129 | 99.62% | 298         | MF765614.1    |
| Bartonella henselae strain GDC21 tmRNA (ssrA) gene, partial sequence | Bartonella henselae | 262          | 472       | 472         | ssrA        | 98%         | 1.00E−128 | 99.61% | 301         | MF765682.1    |
| Bartonella henselae strain GDGZ20 tmRNA (ssrA) gene, partial sequence | Bartonella henselae | 262          | 472       | 472         | ssrA        | 98%         | 1.00E−128 | 99.61% | 297         | MF765628.1    |
| Bartonella henselae isolate Cat_flea_175B 16S-23S ribosomal RNA intergenic spacer, partial sequence | Bartonella henselae | 174          | 270       | 270         | ITS         | 99%         | 2E−68    | 95.40% | 644         | MT095054.1    |
| Bartonella henselae isolate Domestic_cat_151 16S-23S ribosomal RNA intergenic spacer, partial sequence | Bartonella henselae | 174          | 270       | 270         | ITS         | 99%         | 2E−68    | 95.40% | 647         | MT095053.1    |
| Bartonella henselae isolate Domestic_cat_210 16S-23S ribosomal RNA intergenic spacer, partial sequence | Bartonella henselae | 174          | 270       | 270         | ITS         | 99%         | 2E−68    | 95.40% | 648         | MT095050.1    |

A month later, while still administered pradofloxacin-doxycline treatment, physical examination and CBC were normal, with resolution of lymphadenomegaly. A second, aseptic aspiration from the right popliteal and right prescapular lymph nodes was obtained and was negative for the presence of *Bartonella* DNA. Neither anemia nor fever recurred. Consequently, antibiotic treatment was discontinued after 2 months of treatment. Lastly, almost 6 months after the detection of *B. henselae* DNA in affected lymph nodes, and approximately 3 months after cessation of treatment, anemia, fever, and lymphadenomegaly were absent, and a 30%-increase in the cat’s body weight was recorded. Furthermore, a third aspiration from the right and left mandibular, and right popliteal lymph nodes, in addition to an EDTA-anticoagulated blood sample this time, tested negative for the presence of *Bartonella* DNA.

2 | DISCUSSION

The *Bartonellaceae* family comprises over 35 species which infect a wide array of mammalian, reptile, and avian hosts world-wide. Most species demonstrate vector and host specificity, but accidental infections of non-reservoir hosts are possible. Intraerythrocytic colonization enables the bacterium to evade the immune system and facilitates vector-borne transmission. In cats, fleas are the most important blood-sucking arthropods in terms of natural disease transmission. In the present case, heavy flea infestation was noted upon presentation, supporting a possible mode of transmission. Moreover, the detection of *Bartonella* DNA (ssrA and ITS) in affected lymph-nodes with high and first match sequence identity with GenBank deposited *B. henselae* sequences (99.61%-99.62% and 95.40%, respectively) and the apparent response to specific antibacterial treatment, with a negative-PCR result thereafter, all render *B. henselae* the most probable cause of fever and generalized lymphadenitis herein. Whether the infection also instigated the presumptive immune-mediated hemolytic anemia (IMHA), however, remains speculative, since the anemia resolved with immunosuppressive treatment before molecular detection of *B. henselae* and specific antibacterial therapy.

A plethora of clinical conditions are associated with bartonellosis in humans, dogs, and cats, but establishing causality is hindered by the high prevalence of subclinical infections. Additionally, the pathophysiology and clinical ramifications of infection in reservoir hosts (eg, *B. henselae* infection in cats) varies from that in accidental hosts. In cats, seroprevalence ranges from 0% to 71.4% depending on geographic location, age and husbandry conditions, while the prevalence of bacteremia might reach 30% (in the geographic area the present cat came from) and up to 40% in asymptomatic feral cats. Furthermore, infection can persist for months and even years. Experimental-infection of cats with *B. henselae*, *Bartonella claridgeiae*, and *Bartonella koehlerae* is often associated with minimal (eg, self-limiting febrile disease) or absence of clinical disease, notwithstanding cyclic bacteremia and spread of bacteria to many organs, including the liver, kidneys, lymph nodes, myocardium, lung, and brain. Concomitant, lymph node and splenic follicular hyperplasia or lymphocytic inflammation is variably present in histology, but gross necropsy findings and laboratory
The etiological role of Bartonella spp. in the development of fever, lymphadenitis, abnormal vascular growths, encephalitis, uveitis, endocarditis, and myocarditis, among other clinical conditions, is well-established in humans. In cats, despite paucity of studies, fever occurs in naturally-infected cats and in 1, naturally-infected cat with bartonellosis and supraventricular tachycardia, and in an attempt to avoid the development of resistance with monotherapy. Since cats can be silent carriers of Bartonella spp., this human patient, the anemia resolved solely with immunosuppressive therapy, without specific antibacterial treatment, similarly to the present case report. Bartonella henselae can attach and invade mature red blood cells, and thus can predispose to the development of immune and non-immune mediated hemolytic anemia. However, the consequences of B. henselae infection in its natural reservoir host (ie, the cat) might differ from accidental hosts such as humans and whether the infection instigated the presumptive IMHA herein, remains speculative, since the anemia resolved with immunosuppressive treatment before molecular detection of B. henselae and specific antibacterial therapy.

Bartonellosis in cats has been implicated in the development of endocarditis, endomyocarditis-left ventricular endocardial fibrosis complex, myocarditis, and supraventricular tachycardia, which in some cases resolved with antibiotic treatment. In the present case, an association between B. henselae infection and either endocarditis or myocarditis could not be established. Echocardiography failed to demonstrate vegetative valvular lesions in support of the former, while lack of arrhythmias and persistence of gallop rhythm and the heart murmur neither refuted nor confirmed the latter.

Resolution of fever and generalized lymphadenomegaly occurred only after the cat had received pradofloxacin and doxycycline treatment and was accompanied by 2, follow-up negative PCR tests from the lymph nodes, 1 during treatment, and another 3 months after cessation of antibiotic therapy. There is a dearth of clinical studies to define treatment recommendations in feline bartonellosis, and much is based on human studies and in vitro susceptibility tests. Markofloxacin, doxycycline, azithromycin, rifampin, and amoxicillin-clavulanate have all been described, with variable success and development of resistant strains. Pradofloxacin is a novel, extended-spectrum third-generation fluoroquinolone with superior, in vitro efficacy against B. henselae compared to azithromycin and enrofloxacin. In the present case, marbofloxacin/amoxicillin-clavulanate therapy failed to eliminate infection or resolve clinical signs, and a combination of pradofloxacin and doxycycline was eventually administered for 2 months, owing to the aforementioned studies, and in an attempt to avoid the development of resistance with monotherapy. Since cats can be silent carriers of B. henselae, the involvement of other bacterial infections which only responded to pradofloxacin/doxycycline therapy could not have been ruled out, notwithstanding molecular detection of B. henselae in affected, inflamed lymph-nodes, and no evidence for involvement of other organ systems in laboratory tests or imaging studies.

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CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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