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Case Report / *Olgu Sunumu*

Pyogranulomatous myocarditis and diaphragmatic myositis associated with *Bartonella henselae* in a cat from Turkey

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Summary: A case of pyogranulomatous myocarditis and diaphragmatic myositis caused by *Bartonella henselae* in an 18 months old tricolor female cat in Turkey was reported in this study. The cat had flea infestation before clinical disease onset. The cat showed clinical findings including lethargy, anorexia, dyspnea, hypersalivation, and polydipsia. At necropsy, the myocardium contained numerous 1 to 2 mm, discrete to coalescing, white-grey nodular foci with approximately 250 ml of serosanguineous fluid in the thorax. By Warthin-Starry silver staining, argyrophilic pleomorphic coccobacilli were seen within the myocardial and diaphragmatic pyogranulomatous lesions. *B. henselae* DNA was detected in heart, kidney and liver tissues by targeting the riboflavin synthase C (*rib*C) gene by PCR. Using immunofluorescence technique, *B. henselae* was also visualized in myocardial tissues. This report provides additional evidence in support of an etiological association between pyogranulomatous myocarditis and diaphragmatic myositis and *B. henselae* infection in cats.

Keywords: *Bartonella henselae*, cat, diaphragmatic myositis, myocarditis.

Türkiye'de bir kedide *Bartonella henselae* ile ilişkili pyogranülomatöz miyokarditis ve diyaframatik myozitis

Özet: Bu çalışmada Türkiye'de 18 aylık üç renkli dişi bir kedide *Bartonella henselae*'ya bağlı pyogranülomatöz myocarditis ve diyaframatik myositis rapor edilmiştir. Klinik olarak hastalık oluşmadan önce kedide pire enfestasyonu vardı. Kedide durgunluk, iştahsızlık, solunum güçlüğü, aşırı salivasyon ve aşırı su içme isteği semptomları görüldü. Nekropside miyokardda çok sayıda, ayrı ya da birleşme eğilimi gösteren 1-2 mm çapında beyaz-gri nodüller ile göğüs boşluğunda 250 ml serö-sanguinöz yapıda sıvıya rastlandı. Miyokardiyal ve diyaframatik lezyonlarda Warthin-Starry boyama ile argirofilik pleomorfik kokobasiller görülmüştür. Kalp, böbrek ve karaciğer dokularında riboflavin sentaz C (RibC) gen hedefleyen PCR ile *B. henselae* DNA'sı belirlendi. İmmunofloresan teknik ile de myokardiyumda *B. henselae* gösterildi. Bu rapor kedilerde pyogranülomatöz myokarditis, diaframatik myozitis ve *B. henselae* enfeksiyonu arasındaki etiyolojik ilişkiyi destekleyen ilave bir kanıt sağlamaktadır.

Anahtar sözcükler: *Bartonella henselae*, diyaframatik myozitis, kedi, miyokarditis.

Bartonella henselae is an important emerging pathogen in animals (2) and people (15). Numerous domestic animals species, including cats, can be chronically infected; thereby serving as reservoir hosts for transmission through arthropods or hosts (1,2). Cats infected with *B. henselae* may manifest clinical abnormalities, associated with endocarditis, myocarditis, lymphadenopathy, meningitis, radiculitis and reproductive disorders. Recently, using molecular and immunohistochemical techniques, *B. henselae* infection was confirmed in two cats from the United States with pyogranulomatous myocarditis and diaphragmatic myositis (18).

In this report, we used histochemical, immunohistochemical, and PCR techniques to confirm *B. henselae* infection in a cat from Turkey with pyogranulomatous myocarditis and diaphragmatic myositis.

An 18-month-old intact female tricolor cat was submitted to Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Pathology for necropsy. The owner had two indoor flea-infested cats of

the same age. Following treatment for the flea infestations, one of them became ill and showed clinical findings including lethargy, loss of appetite, labored breathing, dyspnea, excessive salivation, and polydipsia. After a period of time, the ill cat died whereas the other remained clinically healthy.

At necropsy, the thorax contained approximately 250 ml of serosanguineous fluid. The right ventricle was dilated and the myocardium contained numerous 1 to 2 mm, discrete to coalescing, white-grey nodular foci some of which were slightly raised above the epicardial surface (Figure 1). Gross lesions were not visible in the diaphragm. The lungs were normal in appearance with small foci of atelectasis. Mediastinal and retropharyngeal lymph nodes were enlarged. Tissue samples were fixed in 10% neutral buffered formalin, and additional unfixed tissues including heart, liver, and kidney were stored at -30 °C for PCR analysis. Paraffin-embedded sections were stained with hematoxylin and eosin (HE), Warthin-Starry silver impregnation, and Brown-Brenn gram, Periodic acid-Schiff, and Giemsa.

Microscopically, the myocardial and diaphragmatic lesions consisted of multifocal to coalescing pyogranulomas of varying sizes, with fragmented and lysed myofibrils among the inflammatory foci (Figure 2). Regional lymph nodes contained discrete necrotic foci in the hyperplastic lymph follicles with the germinal center and a small number of reactive macrophages lightly scattered on the periphery of the necrotic foci and within the medullary sinuses. The liver was markedly congested with mild hepatocyte centrilobular fatty change. Warthin-Starry silver stain revealed clusters of coccobacilli within pyogranulomatous lesions in both the diaphragm and myocardium (Figure 3). Warthin-Starry stains of the lymph nodes, liver, lung, and kidney were negative. Other special stains of myocardial and diaphragmatic tissue sections for fungi and bacteria were also negative.

Bartonella immunofluorescence staining was carried out in the Intracellular Pathogens Research Laboratory, College of Veterinary Medicine, North Carolina State University and performed on 5µm sections of paraffin-embedded myocardium and diaphragm on poly-L-lysine coated slides as previously described with certain modifications (7). Slides were deparaffinized and rehydrated using graded ethanol washings, and rinsed in PBS and 0.02% Tween 20 (PBST). Following deparaffinization and rehydration, slides were subjected to heat-induced epitope retrieval; briefly, slides were immersed in sodium citrate buffer (pH 6.0) and placed in a decloaking chamber at 115°C for 30 seconds. After blocking overnight with Dako Protein Block serum free (Dako, Carpinteria, CA), the slides were incubated at 4°C for 1 hour with 1:50 dilution of mouse monoclonal antibody against *B. henselae* (Cat#ab704, Abcam, Cambridge, MA) in Dako Antibody diluent (Dako). After incubation with the primary antibody, immunodetection was carried out using a secondary goat anti-mouse immunoglobulin tagged with a fluorophore (Cy-3 Conjugate) (Cat#115-165-003, Jackson Laboratories, Bar Harbor, ME) diluted (1:1000) in Dako Antibody diluent (Dako) at 4 °C for 20 minutes in the dark. After incubation, slides were washed 5 times for 5 minutes per wash using PBST. Tissues sections were dried and mounted with Vectashield containing DAPI (Vector Laboratories Inc., Burlingame, CA). Slides were imaged on an epifluorescent microscope under 60X (oil immersion) magnification for 20 fields. Formalin-fixed canine lung tissue infected with *B. henselae* was used as a positive control. In the myocardium, reddish-orange fluorescing *B. henselae* organisms were visualized as discrete clusters, whereas *B. henselae* immunoreactivity was not found in three independently processed sections of the diaphragm (Figure 4).

Genomic DNA was extracted from frozen heart, liver and kidney tissue samples using commercial DNA extraction kit (Invitrogen, Thermo Fischer Scientific, Waltham, MA) according to a previous study (17) and the manufacturer's instructions. The tissues were tested for *Bartonella* spp. with primers (BARTON-1: 5'-TAACCG ATATTGGTTGTGTTGAAG-3' and BARTON-2: 5'-TAAAGCTAGAAAGTCTGGCAACATAACG-3') targeting the riboflavin synthase gene (ribC) (10). Using a 1.5% agarose gel, the amplified DNA fragments from all three tissues were 588 bp in size, consistent with *B. henselae* (Figure 5).

There is increasing evidence that *B. henselae* can cause myocarditis in cats. Myocarditis and endocarditis have been reported in both experimental (11,13) and naturally-infected cats (6,12,18). Based upon histochemical, immunohistochemical and PCR evidence, *B. henselae* infection was confirmed in this cat from Samsun, Turkey that died of pyogranulomatous myocarditis and diaphragmatic myositis. In 1993, Pedersen et al. (14) described feline "infectious granulomatous myocarditis/diaphragmitis" in cats from California. Despite an intensive investigation involving naturally-infected cats and attempts to transmit a putative transmissible agent to experimentally-infected cats, no etiological agent was found. Following intraperitoneal inoculation of blood from an affected cat to specific pathogen-free cats, pathologically similar granulomatous lesions developed in the heart and diaphragm. Microbiological cultures, electron microscopy, and Warthin-Starry staining were negative (14). Subsequently, based upon molecular and immunohistochemical findings, pyogranulomatous myocarditis and diaphragmatic myositis were reported in two cats from North Carolina that were naturally-infected with *B. henselae* (18). The

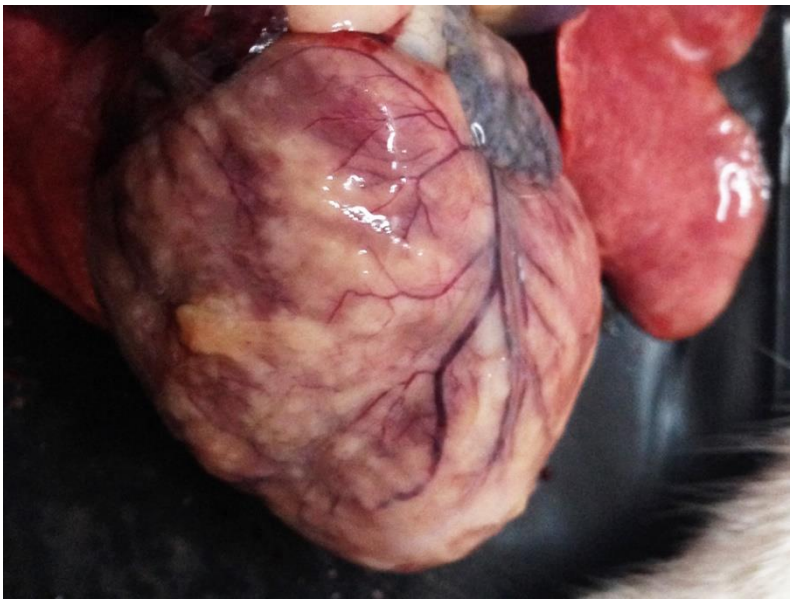

Figure 1. Numerous, discrete to coalescing, white-gray, pin-point nodules throughout the heart. Some nodules bulged slightly from the epicardial surfaces.
Şekil 1. Kalbin tüm yüzeyini kaplayan çok sayıda, ayrı ya da birleşen beyaz-gri, toplu iğne başı büyüklüğünde nodüller. Bazıları epikardiyal yüzeyden hafifçe kabartı oluşturmaktadır.

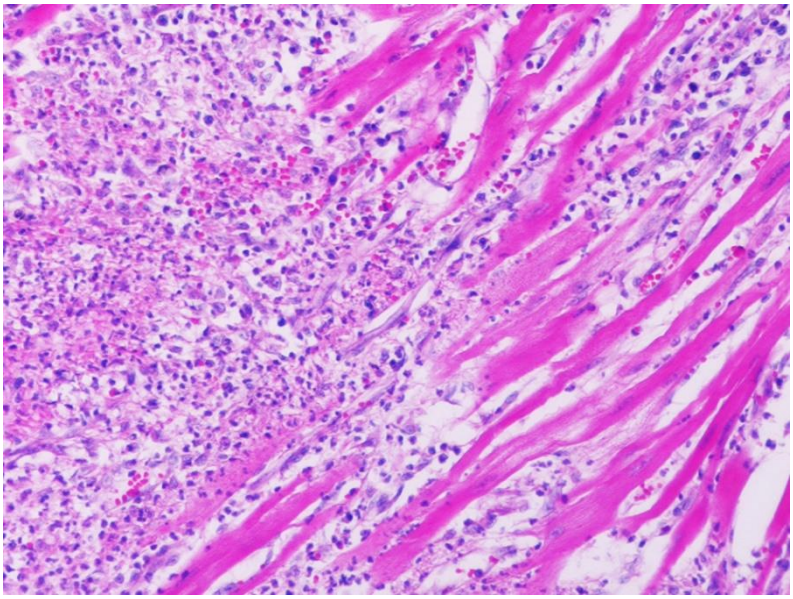

Figure 2. Pyogranulomatous myocarditis, including macrophages admixed with neutrophils among intact or degenerated myofibrils. HE. 240X.
Şekil 2. Sağlam ya da dejenere myofibriller arasında nötrofiller ve makrofajları içeren pyogranülomatöz miyokarditis. HE. 240X.

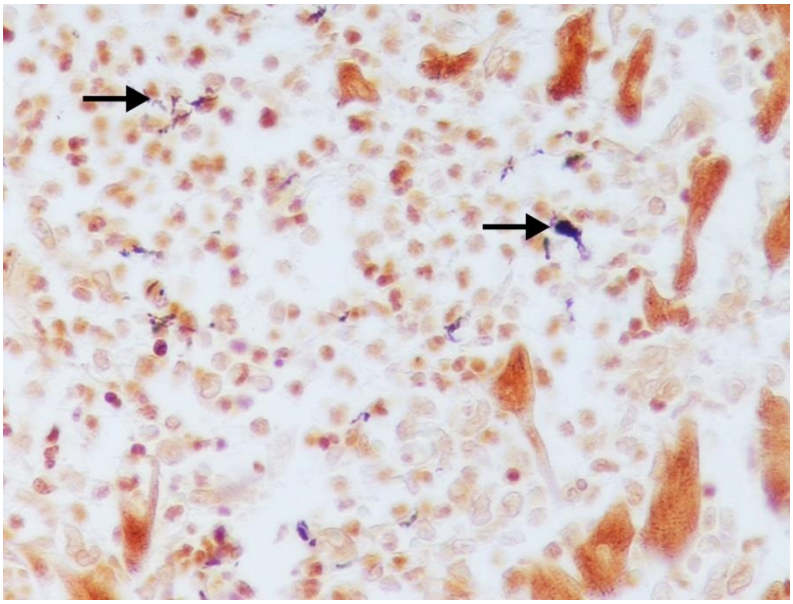

Figure 3. By Warthin-Starry silver stain, black short rod-shaped bacilli visualized as individual cells or clusters (arrows) within pyogranulomatous lesions in diaphragmatic muscle. 400X.
Şekil 3. Wartin-Starry gümüş boyamada diyafram kasındaki pyogranülomatöz lezyonda tek tek ya da küme halinde siyah çubuk şekilli basiller (Oklar). 400X.

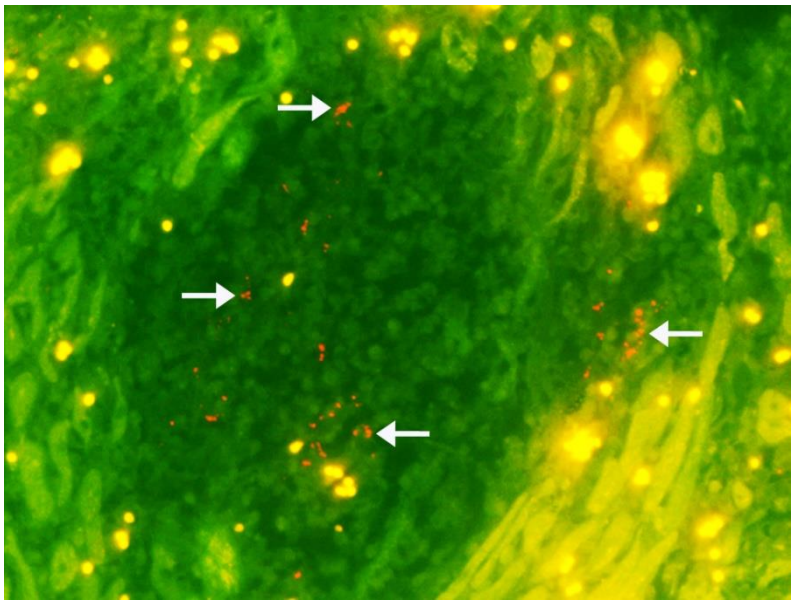

Figure 4. Under oil immersion (60X objective) bacteria are visualized in clusters in the myocardium with reddish-orange fluorescence (arrows) using a *B. henselae* monoclonal antibody and a secondary antibody labelled with Cy3.
Şekil 4. İmmersiyon büyütmede (60X), *B. henselae* monoklonal antikoru ve Cy3 ile işaretli sekonder antikor kullanarak miyokardiyumda kırmızı-turuncu floresan renkte bakteri kümeleri (oklar) gösterilmektedir.

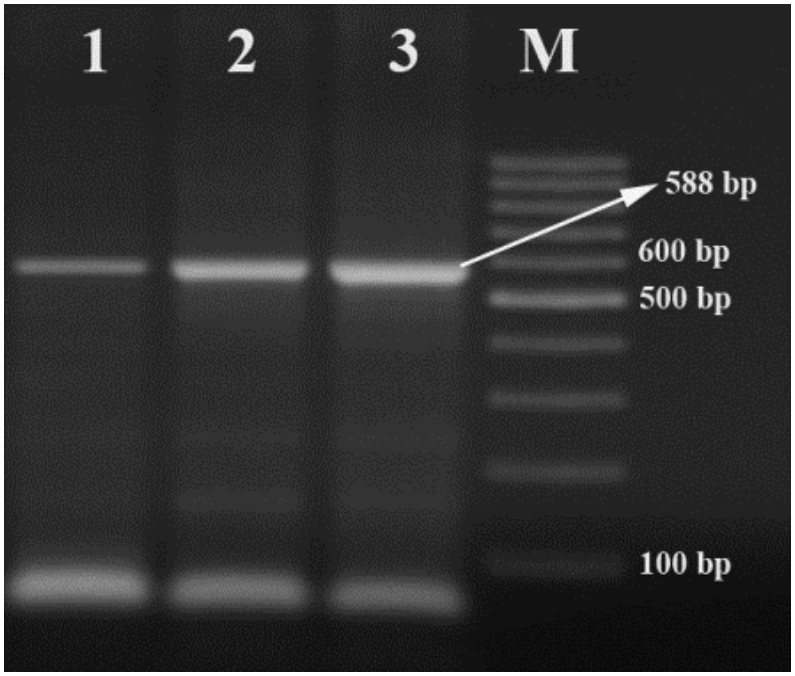

Figure 5. *B. henselae* PCR amplification from kidney (Lane 1), heart (Lane 2), liver (Lane 3), M: marker (100-1000 bp).
Şekil 5. *B. henselae*'nin PCR sonuçları. 1: Böbrek; 2: Kalp; 3: Karaciğer; M: İşaretleyici (100-1000 bp).

gross and histopathological findings in the myocardium and diaphragm of these two previous reports from North America were similar to the pathology found in this cat from Turkey. In the context of comparative pathology (3), granulomatous myocarditis has also been documented in dogs and humans infected with *B. henselae.* It is unknown if *B. henselae* affects other striated muscles, but based upon cases from children (16), the bacterium may have a tropism for other striated muscles, apart from myocardium and diaphragm. Unfortunately, other striated muscles were not collected from this cat during necropsy.

Due to diagnostic limitations associated with microbiological testing, determining the infectious cause of pyogranulomatous lesions can be challenging. Based on these cases, infection with a *Bartonella* spp. should be included in the differential diagnostic considerations for cats with pyogranulomatous myocarditis and diaphragmatic myositis. Although other parenchymal organs and nervous tissues were examined, pyogranulomatous lesions were found only in the myocardium and diaphragmatic muscle of this cat. Pathologically similar, but more widely distributed pyogranulomatous lesions can be observed in parenchymal tissues in cats with feline infectious peritonitis (FIP), actinomycosis, nocardiosis, mycobacterial and systemic mycotic infections (8). Because the pyogranulomatous lesions in this cat were restricted to myocardium and diaphragm, FIP virus testing

was not performed. Special stains failed to support other bacterial or fungal etiologies. As granulomatous myocarditis has now been reported in cats, dogs, and humans in association with *B. hensleae* infections, the recently proposed postulate of comparative infectious disease causation has been satisfied for this lesion (2).

In this cat, bacteria were visualized by Warthin-Starry stain of myocardium and diaphragm, by *B. henselae* immunofluorescence staining of the myocardium and by PCR amplification from multiple frozen tissues. In humans with cat scratch disease, silver staining was the most sensitive but was less specific than IHC or PCR (16). Discordant results among these three testing modalities are commonly encountered and only infrequently do all 3 testing modalities yield positive results for a given patient (4,18). PCR is more sensitive than IHC, and therefore, molecular testing would be indicated for suspected cases that are IHC negative (4). Although we obtained positive results from all three of these methods, there were inconsistencies in results among the various tissues tested.

This cat had a history of flea infestation prior to the onset of clinical signs. Based on the serological and molecular evidence, there is a high percentage of chronically bacteremic healthy cats infected with *Bartonella* species throughout the world; however, recent evidence suggests that a subset of *B. henselae* strains are more virulent (2). In two surveys from different regions of Turkey, *B. henselae* seroprevalence in cats was 18.8% in Ankara (5) and average 27.5% in the different six provinces (varied from 12.9 to 41.3%) (9). Unfortunately, there are no *Bartonella* seroprevalence studies involving feline populations from the Samsun region.

In conclusion, clarification of the clinical and pathological aspects of *Bartonella* infections in animals and human patients continues to evolve. In cats, concurrent pyogranulomatous lesions in the heart and diaphragm should increase the index of suspicion for *B. henselae* infection. The worldwide emergence of this stealth pathogen has contributed to enhanced awareness among veterinary clinicians and diagnosticians. In the context of One Health, veterinarians should facilitate communications with physicians when bartonellosis is diagnosed in a pet and human illness exists within the same household.

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