

***Bartonella quintana* Endocarditis of the Aortic Valve: First case report in Turkey*****Bartonella quintana* İlişkili Aort Kapak Endokarditi: Türkiye'deki İlk Olgu**H. Selcuk Ozger<sup>1</sup>, Zehra Demirbas Gulmez<sup>1</sup>, Bekir Celebi<sup>2</sup>, İrem Akdemir Kalkan<sup>3</sup>, Murat Dizbay<sup>1</sup><sup>1</sup>Department of Infectious Diseases and Clinical Microbiology, Gazi University School of Medicine, Ankara, Turkey<sup>2</sup>Public Health Institution of Turkey National High-Risk Pathogens Reference Laboratory, Ankara, Turkey<sup>3</sup>Department of Infectious Diseases and Clinical Microbiology, Ankara University School of Medicine, Ankara, Turkey**ABSTRACT**

Endocarditis caused by *Bartonella* strains has been increasingly reported. While a few sporadic case reports associated with *B. henselae* were published in Turkey, no endocarditis cases caused by *Bartonella* spp. have been reported yet. Herein, a case of first *B. quintana* endocarditis in Turkey, diagnosed using molecular methods, was presented.

**Key Words:** *Bartonella quintana*, Endocarditis, Serology, DNA sequence amplification

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*Bartonella* suşlarının neden olduğu endokardit olguları giderek daha fazla sıklıkta rapor edilmektedir. Türkiye'de *B. henselae* ile ilgili birkaç sporadik vaka bildirimini yayınlanmış olmasına karşın, *Bartonella* suşları ile gelişen endokardit olgusu henüz bildirilmemiştir. Bu çalışmada, Türkiye'de moleküler yöntemlerle tanı konan ilk *B. quintana* ilişkili endokardit olgusu sunulmuştur.

**Anahtar Sözcükler:** *Bartonella quintana*, Endocarditis, aortic valve

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## INTRODUCTION

*Bartonella* species are gram-negative, facultative and intracellular bacteria. They can cause bacteremia, endocarditis, trench fever, and bacillary angiomatosis (1). Reported *Bartonella* related infections in humans are associated with *B. hanselae* in Turkey (2-4). *B. quintana* positivity has not been shown in animals and humans in Turkey.

*B. quintana* is responsible for approximately 75% of endocarditis with *Bartonella* species in humans (5). Direct examination, culture, serological and molecular methods (PCR assays) are used in the diagnosis of diseases caused by *Bartonella* species (1). Due to the slow growth of *Bartonella* species, cultures are not routinely recommended for the diagnosis of *Bartonella* infections (1). For the diagnosis of *Bartonella* infections, the gold standard serological test is an immunofluorescence assay (IFA) with a sensitivity of 82% and 93% in people with acute and chronic infection, respectively (1). In recent years, molecular techniques have been used effectively for the identification of *Bartonella* species, and DNA sequence analysis of polymerase chain reaction (PCR) and PCR amplification products of genus-specific gene regions such as *gltA*, *ssrA*, *rib C*, *groEL* have been used for molecular identification of *Bartonella* species (6).

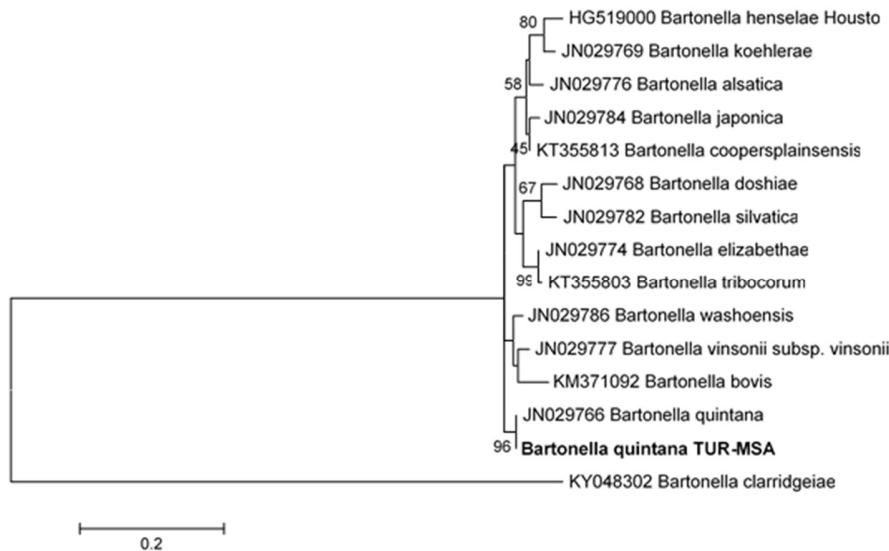
Herein, a case of first *B. quintana* endocarditis in Turkey, diagnosed using serological and molecular methods, was presented.

## CASE REPORT

A 64-year-old Turkish man, from the southeast of Anatolia, was hospitalized with a history of weakness, fatigue and weight loss for seven months. He had a history of diabetes mellitus and hypertension and a moderate aortic regurgitation. In the physical examination of the patient, body temperature, blood pressure, heart rate, and respiratory rate were 36.3°C, 130/63 mmHg, 86/min, 16/min, respectively. A grade 3 diastolic heart murmur was detected at the left upper sternal border. Initial laboratory investigations demonstrated white blood cell count 10 700 cells/micro L (66 % neutrophils ), hemoglobin 9.0 g/ dL aspartate aminotransferase 25 U/L, alanine transaminase 27 U/L, blood urea nitrogen 22 mg/dl, creatinine 0.94 mg/dl, C-reactive protein 85 mg/dl and erythrocyte sedimentation rate: 95 mm/h.

Microscopic hematuria was detected in urinalysis. Transthoracic echocardiography was performed, followed by transesophageal echocardiography. It revealed a severe aortic regurgitation (grade 3) and mobile aortic vegetation measuring 15 x 7 mm on the right coronary leaflet. Roth spots were detected in the ophthalmologic examination. Empirical ampicillin-sulbactam treatment was started after two sets of aerobic and anaerobic blood cultures were obtained. Blood cultures yielded no bacteria. Serological tests for *Coxiella burnetii*, *Brucella* and *Bartonella* spp. were performed to evaluate the etiology of culture-negative endocarditis. An indirect fluorescent antibody test (IFA) was performed in *Bartonella* serology (6). *B. henselae* and *B. quintana* binary antigen closed commercial kit (Focus Diagnostic, California USA) was used for IFA test. IFA test result was positive for *B. henselae* IgG (1/1024 titer) and *B. quintana* IgG (1/1024 titer). Following this result, oral doxycycline and intravenous gentamicin treatment were started. Aortic valve replacement was performed after four weeks of treatment. The tissue was obtained from the aortic valve during the surgery. *Bartonella* was found to be positive in polymerase chain reaction (PCR) testing.

For DNA extraction, the aortic valve was homogenized in a Magnalyser homogenization device (Roche, Rotkreuz, Switzerland) by adding 500µl of phosphate-buffered saline to the tissue sample. 100 µl of the homogenized tissue sample was taken and DNA extraction was performed using the tissue extraction kit (Qiagen, Hilden, Germany). Real-time PCR was performed using *ssrA* gene-specific primer and probe to determine *Bartonella* species (7). The tissue sample was found to be *Bartonella* spp. positive in real-time PCR. Conventional PCR of the *ssrA* gene region and the PCR amplification product were sequenced to determine the species. DNA sequence analysis data were compared with GenBank data using the basic Local Alignment Search Tool (Blast version 2.0). The *ssrA* sequence was 100% compatible with the *B. quintana* strain Toulouse Accession number HG518998.1 and the *B. quintana* strain NCTC12899 Accession number LS483373.1. Using the DNA sequence analysis data and data from other *Bartonella* species at Genbank, the phylogenetic tree was created using the Maximum likelihood statistical method in MAGA 5.1 program and the *B. quintana* Bootstrap phylogeny value was determined to be highly reliable with 96% (Figure 1.)



**Figure 1.** Comparison of *B. quintana* *ssrA* gene amplified from aortic valve tissue with 301 bp sequence data and other *Bartonella* species registered in GenBank with *ssrA* gene data in MEGA5 program. Phylogenetic tree generated by the Kimura 2 parameter model.

Histopathology of the aortic valve showed vegetations and destruction on the valve tissue, with fibrinoid necrosis and inflammation. The patient received gentamicin in combination with doxycycline for two weeks and doxycycline alone

for four weeks. The patient was discharged in a full recovery on the 15th day after surgery, on the 42nd day of the treatment.

## DISCUSSION

This is the first case of aortic valve endocarditis (BCNE) caused by *B. quintana* in Turkey. *Bartonella* spp. are fastidious bacteria that cause blood culture-negative endocarditis (BCNE) and have been increasingly reported (8). The estimated incidence of *Bartonella* spp. endocarditis is ranging from 1.0 to 15.6%, depending on the series (8). *Bartonella* endocarditis has shown worldwide distribution, and a lot of case series of *Bartonella* endocarditis is reported from Europe (8, 9). While a few seroprevalence studies and sporadic case reports associated with *B. henselae* were published in Turkey, no endocarditis cases caused by *Bartonella* spp. have been reported yet (3,4). This might be because of the specific laboratory assessment (serological testing or molecular assays) for *Bartonella* spp. are rarely performed in only a few reference laboratories in our country.

*B. quintana* is responsible for three-fourths of *Bartonella* endocarditis cases (5). Male gender, immunosuppression including HIV infection, alcoholism, previous valvulopathy, and some epidemiologic aspects such as low socioeconomic status or homelessness are defined as risk factors for *B. quintana* endocarditis (5, 8, 9). Our patient had a higher socioeconomic condition. Diabetes mellitus and aortic regurgitation can be considered as risk factors for *Bartonella* endocarditis in our patient. *Bartonella* endocarditis usually presents with prolonged non-specific symptoms such as fever, fatigue, weight loss, or signs of heart failure, such as exertional dyspnea or hypoxia. This may lead to a delay in diagnosis and treatment, as in our patient (9).

Currently, there is a lack of criteria for the diagnosis of *Bartonella* endocarditis (8, 9). Detection of IgG antibodies using the micro immunofluorescence technique has been used for the diagnosis of *Bartonella* endocarditis in many studies (8, 9). *Bartonella* IgG titer of  $\geq 1:800$  is recommended as the cut off for a positive test result (8, 9). However, serological test results, except for *C. burnetii*, are not incorporated into the modified Duke criteria (8, 10). This might be because of the cross-reactivity between various antibodies in serological tests. *Bartonella* serological assays may demonstrate cross-reactivity with Epstein-Barr virus, cytomegalovirus, *Toxoplasma gondii*, *Streptococcus pyogenes*, *Chlamydia* and *Coxiella* (11, 12). Also, cross-reactions can be seen among the *Bartonella* species, as in our case (11, 12). For these reasons, molecular methods, are increasingly utilized to aid in the diagnosis of culture-negative endocarditis. Testing of cardiac valve tissue with *Bartonella* - specific PCR assays is more sensitive than testing blood or serum (8). Amplification of *Bartonella* DNA from the valve tissue has been shown to have high sensitivity and specificity ranging from 72 – 98% (9). Edouard et al. (8) suggested that a positive PCR result from a valvular biopsy specimen can be considered as a definitive criterion for *Bartonella* endocarditis.

Histopathology can confirm the diagnosis by showing valvular inflammation and remains the gold standard for the diagnosis of endocarditis (8, 9). Histopathological examination is generally non-specific in *Bartonella* endocarditis and primarily shows chronic inflammation with macrophage and lymphocytic infiltration (8-10). In our patient, in addition to serological test results, chronic inflammation with marked fibrosis and PCR positivity in valvular valve tissue were detected. The diagnosis was confirmed with all diagnostic steps suggested above.

## CONCLUSION

*Bartonella* spp is fastidious bacteria that cause BCNE and have been increasingly reported. Due to the lack of diagnostic criteria, different methods should be used for diagnosis. The combination of serological testing and DNA sequence analysis of PCR increases the chance of diagnosing bartonella-related endocarditis.

## Conflict of interest

No conflict of interest was declared by the authors.

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