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Case Report

Multispacer sequence typing of *Coxiella burnetii* DNA from removed prosthetic heart valve material discloses first human case of infective endocarditis caused by MST_18

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ABSTRACT

Introduction: In Denmark, Q fever has previously been considered a rare and imported disease; however, recent testing of antibodies in cattle as well as humans has indicated that the infection is widespread. A 76-year-old Danish man was diagnosed with infective endocarditis and underwent open surgical aortic valve replacement with insertion of a biological valve. Due to paravalvular leakage, destruction of the aortic annulus, and an aortic root abscess, the patient underwent re-operation 3 weeks later, with replacement of the biological valve and insertion of an aortic prosthetic tube. Despite treatment with various broad-spectrum antibiotic regimes, the patient died 3.5 months after initial hospital admission.
Methods: The causative agent was probed by PCR amplification of bacterial DNA on the removed prosthetic aortic valve using broad range primers targeting the variable regions V1–V3 of the 16S RNA gene. After identification of *Coxiella burnetii*, multispacer sequence typing (MST) was performed by PCR amplification of 10 intergenic sequences.
Results: BLAST analysis of DNA from prosthetic valve material identified a 16S rRNA gene fragment almost identical to the type strain of *C. burnetii* (462/463 nt). Molecular typing allocated the strain to MST_18.
Conclusions: Molecular methods are increasingly used to characterize isolates and to determine relationships between isolates that cause disease in different contexts and geographical areas. The present case demonstrates that identification and typing of *C. burnetii* is achievable without access to biosafety level 3 containment and highlights the first molecular characterization of an uncultured strain of *C. burnetii* causing infective endocarditis.

Introduction

Among numerous microorganisms associated with culture-negative infective endocarditis (IE) is *Coxiella burnetii*, a Gram-negative, facultative anaerobic, intracellular pathogen causing Q fever (Lamas et al., 2016; Grisoli et al., 2014). Culture of *C. burnetii* is achievable utilizing specific conditions in biosafety 3 containment, but serological testing is essential for routine diagnosis. Most studies addressing *C. burnetii* have been from regions with a particular interest in the infection or have been aimed at clarifying local epidemics, such as the recent outbreak in the Netherlands (Kampschreur et al., 2014; Eldin et al., 2017). In Denmark, Q fever has previously been considered a rare and imported disease; however, studies have revealed a seroprevalence of *C. burnetii* antibodies of 59% in dairy herds and 11% among people with relevant exposure to domestic animals (Agger et al., 2010; Bosnjak et al., 2010; Bacci et al., 2012). These studies dismiss the assumption of low seroprevalence in Denmark, but may also reflect a rising interest in *C. burnetii* rather than the emergence of a new zoonosis.

C. burnetii infects a large number of animals and can be found in urine, manure, wool, milk, mammary glands, uterus, placenta, and abortion products. *C. burnetii* can travel large distances by wind, and the inhalation of contaminated aerosols from domestic ruminants is considered the predominant route of transmission to humans.

The clinical picture of acute Q fever ranges from asymptomatic or influenza-like symptoms to severe pneumonia and hepatitis. Immunocompromised patients, pregnant women, and patients with structural cardiovascular abnormalities are at risk of a more severe course of infection.

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The persistence of *C. burnetii* in the host may signify progression to IE, which is the most frequently reported form of persistent *C. burnetii* infection (Eldin et al., 2017).

Case

A 76-year-old Caucasian male with ischemic heart disease, a pacemaker, and a prosthetic aortic valve was admitted with a history of prolonged fever, fatigue, and dry cough. Transesophageal echocardiography (TEE) showed no indication of IE, but a computed tomography scan of the cerebrum revealed multiple small infarcts that were assumed to have originated from a cardiac embolic source.

The patient's clinical condition deteriorated, and further TEE 3 weeks later revealed a loose prosthetic aortic valve with possible excrescences. The patient underwent open surgical aortic valve replacement with the insertion of a biological valve. Blood cultures as well as culture of the removed valve were negative.

PCR amplification of bacterial DNA was performed on the removed prosthetic aortic valve using broad range primers targeting the variable regions V1–V3 of the 16S RNA gene (Kemp et al., 2013). The 16S sequence was 99% similar (462/463 nt) to *C. burnetii* strain ATCC VR-615 (NCBI reference sequence NR_104916).

TEE at 2 weeks postoperative revealed a paravalvular leakage, destruction of the aortic annulus, and an aortic root abscess, and the patient underwent re-operation with replacement of the biological valve and insertion of an aortic prosthetic tube.

Culture and 16S RNA gene PCR on the second removed valve were negative, but Coxiella serology was positive, with extremely high titers (IgM phase I, 1024 (cutoff $\geq$ 128); IgM phase II, 1024 (cutoff $\geq$ 256); IgG phase I, 512.000 (cutoff $\geq$ 512); IgG phase II, 512.000 (cutoff $\geq$ 1024)).

Five weeks after the second operation, a renewed TTE (transthoracic echocardiography) disclosed apical dyskinesia with an ejection fraction of 40–50%. The patient's condition deteriorated and TEE indicated perforation of the frontal mitral valve. Further surgical treatment was deemed inappropriate, and the patient died 3.5 months after initial hospital admission.

At autopsy, no vegetations were macroscopically visible on the cardiac valves, and culture and 16S rRNA gene PCR on valve material were negative.

The antibiotic regimes included meropenem, gentamicin, vancomycin, echinocandin, and metronidazole, as well as rifampicin and doxycycline when *C. burnetii* was identified on the removed prosthetic aortic valve.

Discussion

Valvular prostheses are pivotal risk factors for *C. burnetii* IE. Lesions are characterized by fibrosis and calcification, and insignificant inflammation and small or absent cardiac vegetations are probably due to the intracellular nature of the bacterium. Thus, the diagnosis of *C. burnetii* IE is difficult. A large single-center study spanning 26 years was only able to demonstrate valvular vegetations by echocardiography in 30% of cases of confirmed IE according to the Duke criteria (Million et al., 2010).

Due to the high infectivity, culture of *C. burnetii* is restricted to biosafety level 3 containment, and isolation of the microorganism is rarely included in standard diagnostic procedures. *C. burnetii* expresses two antigens (phase I and II), and immunofluorescent detection of both phase I and phase II antibodies is considered the gold standard. PCR is a valuable diagnostic tool in acute as well as chronic infection. Molecular methods are increasingly used to characterize isolates and to associate isolates that cause disease in different contexts and geographical areas (Chmielewski et al., 2009; González-Barrio et al., 2016). Plasmids appear to be ubiquitously present in *C. burnetii*, and three different plasmid types are reported. While the QpDV plasmid type was shown to be more frequent in isolates associated with human abortion, this was not the case for IE (Angelakis et al., 2013). Genotyping using multispacer sequence typing (MST) is based on the variability of 10 intergenic sequences. MST uses standardized nomenclature, and the publicly accessible database currently encompasses 49 MST types identified at all 10 loci.

Prosthetic heart valve material from the present case was minced and treated with lysis buffer and proteinase K, and DNA was purified using the MagNA Pure Compact System (Roche) for tissue culture as recommended by the manufacturer. After detection of *C. burnetii* by 16S rRNA gene PCR, approximately 30 μl (22 ng DNA/μl) was kept at −20 °C. MST was performed as described by Glazunova et al. (2005), except that the volume of each PCR reaction was reduced to 12.5 μl including 1 μl template. Due to truncated sequence reads, PCR and sequencing was repeated for five spacers, after which alleles could be precisely determined for all 10 spacers. The case presented here could be allocated to MST_18. Nineteen MST_18 isolates are listed in the Mediterranean Infection Database (Anon, 2018): nine cultured from human blood, urine, or placenta, and 10 from goats, sheep, and cattle. The Danish case constitutes the first *C. burnetii* IE case sequence-typed directly from removed prosthetic material, and the first reported case of MST_18 associated with IE (Figure 1).

The treatment of *C. burnetii* IE is challenging and requires long-term supplementation with rifampicin or hydroxychloroquine (18 months for native valves and 24 months for prosthetic valves) (Million et al., 2010). Due to earlier diagnosis and appropriate dual antibiotic therapy, the prognosis of *C. burnetii* endocarditis has improved. One study reported a mortality rate of 9.3% for *C. burnetii* endocarditis, with an acute presentation (severe endocarditis) being associated with a poor outcome (Kampschreur et al., 2014).

Mortality rates of about 5% at 3 years have been reported, and factors independently associated with death were age, stroke, and prosthetic valve at diagnosis. It has been recommended that systematic echocardiography should be performed in all patients

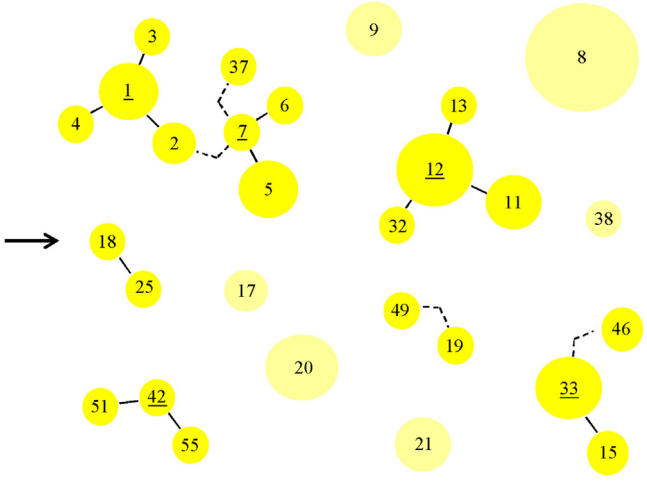

Figure 1. Clonal relationship of *Coxiella burnetii* multispacer sequence types (MSTs) associated with infective endocarditis (28 MSTs encompassing 1–38 isolates). MSTs of 95 human heart valve isolates and 14 valvular prosthesis isolates were downloaded from the Mediterranean Infection Database (Anon, 2018) and supplemented with the present strain of MST_18 (arrow). Distances between nodes were calculated by Phyloviz (https://online.phyloviz.net/index). Single locus variants are connected with solid lines, double locus variants are linked with dotted lines. Putative founders or core MSTs are underlined. The size of the node represents the number of strains.

diagnosed with primary Q fever to detect any predisposing valvular lesions and, if such lesions are detected, that regular monitoring and prophylactic treatment with doxycycline and hydroxychloroquine should be provided (Eldin et al., 2017; Million et al., 2010).

In the case presented here, there was no known exposure to domestic animals, or to extensive travel. This emphasizes the importance of clinical awareness of this unexpected diagnosis of an infection requiring complex treatment.

The first report on the rapid diagnosis of Q fever endocarditis by PCR of aortic valve tissue was recently published (Kumpf et al., 2016), and a retrospective review of resected cardiac valves/prostheses from 6401 patients undergoing valve surgery led to an unexpected diagnosis of latent Q fever endocarditis in 14 cases (0.2%) (Grisoli et al., 2014). The case reported here highlights the possibility of identifying and typing *C. burnetii* in clinical microbiology laboratories without access to biosafety level 3 containment. This appears to be the first case with molecular characterization of an uncultured strain of *C. burnetii* causing IE.

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Ethical approval

Not applicable.

Conflict of interest

We declare no competing interests.

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