Case Report

Infective endocarditis caused by *Bartonella quintana* in Greenland

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Introduction: Bartonellosis has not been described previously in Greenland. Here, we present a case of infective endocarditis due to *Bartonella quintana* in a patient living in western Greenland.

Case presentation: A 68-year-old man from Disko Island in western Greenland underwent aortic valve replacement because of infective endocarditis. Culture from blood and tissue was negative. PCR of bacterial DNA and DNA sequencing revealed DNA from *B. quintana* in the valve, and the finding was confirmed by species-specific PCR.

Conclusion: This case demonstrates the presence of *B. quintana* in Greenland. The finding emphasizes the importance of testing for unexpected agents when culture fails to identify the cause of a severe disease.

Keywords: *Bartonella quintana*; ciprofloxacin; DNA sequencing; infective endocarditis; PCR; tetracyclin.

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Introduction

Detection of bacterial DNA by PCR and subsequent species identification by DNA sequencing of the PCR product has in many places become an effective tool for establishing the causes of culture-negative infections. In particular, in infective endocarditis culture from blood and surgically removed cardiac valve material may be negative either due to antibiotic treatment prior to sampling or because the infection is caused by non-cultivable bacteria such as *Bartonella* spp. and *Coxiella burnetii*.

PCR and DNA sequencing have been used successfully in establishing *C. burnetii* as an agent of infective endocarditis in eastern Greenland, an area previously not known as being endemic for Q fever (Koch *et al.*, 2010). In the case presented here, PCR and DNA sequencing were used to demonstrate *Bartonella quintana* as the cause of infective endocarditis in a patient from western Greenland. A fairly high proportion of cases of infective endocarditis in Greenland are negative by culture (Madsen *et al.* 2009), and Q fever and bartonellosis may be more common in Greenland than previously anticipated.

Case story

In July 2009, a 68-year-old severely obese man living at Disko Island in western Greenland was referred to Copenhagen University Hospital (Rigshospitalet) because of severe aortic insufficiency and a weight loss of more than 30 kg. Except for treatment with metformin and glimepiride for non-insulin-dependent diabetes mellitus, enalapril for hypertension, and simvastatin for hypercholesterolaemia for 1 year, the patient had no previous history of diseases. The patient had no history of travelling, except for a few short trips to family in Denmark, and had no excessive alcohol consumption. A temporary pacemaker was inserted due to third-degree A-V block. Because of continuously elevated C-reactive protein of 37–141 mg ml⁻¹ and leucocyte counts of more than 5 × 10¹⁰ l⁻¹, diagnostic procedures were repeatedly postponed. The patient was initially treated with cefuroxime, which was supplemented with fusidic acid after 6 days, ciprofloxacin after 8 days, and meropenem and vancomycin after 13 days, without any effect on the laboratory test results. Twenty days after admission, transoesophageal echocardiography showed several vegetations on the aorta valve, and a diagnosis of infective endocarditis was established.

Repeated blood cultures were negative. The patient underwent aortic valve replacement with insertion of an aortic homograft, i.e. a bioprosthesis. No bacteria could be cultured by routine culture from the affected valve. The patient was started on antibiotic treatment with meropenem, fusidic acid and ciprofloxacin for infective endocarditis of unknown aetiology. Ciprofloxacin was chosen instead of gentamicin due to impaired renal function.

A piece of the removed aortic valve was analysed by PCR for part of the bacterial 16S rRNA gene and DNA sequencing was carried out as described previously (Gleesen *et al.*, 2008). DNA from *B. quintana* was detected with 450/450 identical bases when compared with sequences in GenBank.

The species identification was confirmed using a species-specific PCR modified from a previously published assay using the same primers (Bereswill *et al.*, 1999). The PCR was performed with 50 µl reaction volumes using 1 µl Hot-StarTaq master mix (Qiagen) with 0.4 µM (final concentration) of each primer. The conditions of the PCR specific for *Bartonella henselae* were as follows: 95 °C for 15 min and 40 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s. The conditions for the *B. Quintana*-specific PCR were: 95 °C for 15 min, followed by 39 cycles of 94 °C for 30 s, 49 °C for 30 s and 72 °C for 30 s. Both PCRs were performed with a DNA Engine Dyad cycler (Bio-Rad). The PCR products were analysed by 2 % agarose gel electrophoresis after staining with ethidium bromide. The PCR products were 393 and 390 bp for *B. henselae* and *B. Quintana*, respectively.

Subsequently IgG antibodies to *B. quintana* were found in the patient's serum with a titre of 4096 (cut-off value of 256), whereas IgM antibodies were undetectable.

The patient was treated with doxycycline and ciprofloxacin with doses adjusted to the patient's severe obesity for 6 months and the recovery was uneventful. A permanent pacemaker was implanted 2 months after admission. On follow-up by telephone interviews 1 and 2 years after the surgery, the patient reported feeling well and living his life as he did before the disease.

Discussion

Infections caused by *B. quintana* include trench fever and infective endocarditis. The bacterium is transmitted from person to person by body lice and the disease is associated with poor social and hygiene conditions. In the case described here, no evidence of particularly poor living conditions was revealed from the history of the patient. Neither the patient nor anybody close to him had ever noticed body lice. Body lice have been found in western Greenland in archaeological excavations of Viking settlements (Sadler, 1990). Very few cases of endocarditis caused by *B. quintana* have been reported from Nordic countries (Jalava *et al.*, 1995; Vento *et al.*, 2008; Ehrenborg *et al.*,

2009). The seroprevalence of *B. quintana* is generally very low in Scandinavia (McGill *et al.* 2005), and not even in risk groups with higher seroprevalence is trench fever observed (Ehrenborg *et al.*, 2008).

As *B. quintana* does not grow *in vitro* on standard culture media, infections by the bacterium could previously be demonstrated only by detection of specific antibodies in serum from the patient. In areas where bartonellosis is not common, suspicion of *B. quintana* infection is low, and the risk of not recognizing it in individual cases is high. The present case illustrates how infections with unexpected and non-cultivable bacteria may lead to diagnostic delay and treatment with antibiotics without an effect on the causative microorganism. The development of PCR for bacterial DNA with subsequent DNA sequencing as a method of identification of non-cultivable bacteria has made detection of unexpected non-cultivable pathogens possible. In the present case, the finding of a completely unexpected bacterium, *B. quintana*, was confirmed by species-specific PCR. The identity of the bacterium was confirmed using a PCR method adapted as a part of the Danish anti-bioterrorism programme (Kemp *et al.*, 2012).

A previous study on a limited number of patients in Greenland showed a high frequency of cases of infective endocarditis due to *Streptococcus pneumoniae* (Madsen *et al.*, 2009). In many of the patients, no bacteria could be detected by routine culture. Although this may largely be explained by difficulties in carrying out diagnostic procedures due to the special logistic challenges in this large and scarcely populated country, a significant number of culture-negative cases of infective endocarditis may be due to non-cultivable bacteria. Testing for antibodies to *Coxiella burnetii* and *Bartonella* spp. should be considered in culture-negative infective endocarditis in Greenland.

In conclusion, *B. quintana* was demonstrated as the cause of infective endocarditis in a patient from Greenland. The finding was unexpected, as bartonellosis has not been recognized in this area. This case illustrates how routine use of culture-independent diagnostic techniques such as PCR and DNA sequencing adds new information to the knowledge of the epidemiology of infectious diseases.

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