**Case Report**

A 29-year-old Australian man was admitted in April 2012 for elective aortic and mitral valve replacement for progressive severe aortic and mitral regurgitation with mild aortic root dilatation from Marfan’s syndrome. His past medical history included a meticillin-susceptible *Staphylococcus aureus*-colonized chronic left leg ulcer requiring oral flucloxacillin 500 mg four-times daily, chronic fatigue syndrome and recent partial dental clearance. He denied intravenous drug use. He lived in a country town where he worked as a house painter.

A St Jude mitral and aortic valve replacement was performed with preservation of the aortic root. An intra-operative diagnosis of native valve infective endocarditis was suspected due to the appearance of concurrent healed aortic and mitral valve vegetations. Interestingly, his pre-operative transoesophageal echocardiogram was normal.

Post-operatively, further history of occupational and environmental exposures relevant to endocarditis was obtained. Cat scratches, farm animal exposure, ingestion of unpasteurized milk and abattoir work raised the possibility of *Bartonella* spp., *Brucella* spp. and *Coxiella burnetii* (Q fever) as infectious aetiologies. Twelve years earlier (late March 2000), he commenced work at a local abattoir. At commencement of employment, he underwent a medical review and was screened for Q fever on 28 March 2000; baseline serology was negative (immunofluorescence phase II antibody <10, complement fixation test against phase II antigen <2.5), and a skin test using 0.5 ml purified killed suspension of *C. burnetii* was also negative. Documentation illustrates that he was subsequently vaccinated 7 days later with Q-Vax (CSL Biotherapies Q fever vaccine 0.5 ml containing 25 μg purified killed suspension of *C. burnetii*). He worked at the abattoir for 8 months. Approximately 9 months later, he reported to his local medical officer with unexplained loss of weight (30 kg) and fatigue. In the intervening years prior to cardiac surgery, he also reported intermittent drenching night sweats; however, *C. burnetii* serology was not performed nor did he receive any antibiotic treatment other than flucloxacillin for his leg ulcer.

Physical examination was remarkable for marfanoid features and chronic venous insufficiency of his left leg without active infection. An orthopantomogram excluded an odontogenic source of infection. Blood cultures performed intra- and post-operatively revealed *Staphylococcus hominis* in 1 of 12 bottles; this was judged to represent contamination, and other culture bottles remained negative with extended incubation to 21 days. There were polymorphonuclear leukocytes on aortic and mitral valve tissue, but Gram stains on fresh samples were negative, and there was no growth on standard media despite prolonged incubation.

Aortic valve histology demonstrated macroscopic areas of calcification without obvious vegetations. Microscopically there were features of nodular fibrocalfic degeneration in addition to a mixed inflammatory infiltrate including neutrophils and histiocytes with associated fibrin-rich exudate and reactive fibroblast proliferation (Fig. 1a–c). Histopathology demonstrated numerous aggregates of Gram-negative cocco-bacilliform bacteria in areas of active inflammation, which were Warthin–Starry (Fig. 1d) and Giemsa stain positive. No fungal elements were identified using periodic acid–Schiff or Grocott’s methenamine silver stains, and no acid-fast bacilli were detected using

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**Coxiella burnetii** endocarditis after Q fever vaccination

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**Coxiella burnetii** is the causative bacterium of Q fever, a vaccine-preventable infection. *C. burnetii* is an unusual cause of culture-negative endocarditis. Here, we present a case of Q fever native valve endocarditis that developed in a young man despite prior vaccination. Definitive diagnosis was difficult and required *C. burnetii*-specific PCR testing.
Ziehl–Neelsen or Wade–Fite stains. The chordae tendineae of the mitral valve had yellow polypoid nodules ranging from 1 mm to 3 mm without obvious vegetations. There was fibro-myxoikel degeneration with calcification, but no inflammatory features.

These findings raised the possibility of *Bartonella henselae* or *C. burnetii* as causative pathogens, and serology was performed for a range of fastidious Gram-negative or intracellular organisms. Positive results were recorded for *Legionella micdadei* (titre 4096), *Bartonella henselae* (titre 1024), *Rickettsia* spp. and *Mycoplasma pneumoniae* (titre 80) (Table 1). Q fever serology was strongly suggestive of chronic infection (negative IgM phase I and phase II antibody, positive IgA and IgG phase I and phase II antibody with titres >3200) (Table 1). Moreover, the titre was consistent with endocarditis as per the modified Duke criteria (phase I antibody >1: 800) (Vollmer et al., 2010).

Broad-range bacterial 16S rRNA gene PCR testing performed on aortic valve tissue yielded a weak positive result, but was insufficient to perform sequencing. No bacterial-specific PCR products were identified from mitral valve tissue. Organism-specific PCRs were negative for *Bartonella henselae, L. micdadei* and *Legionella* spp. for the aortic and mitral valves. However, specific PCR products for *C. burnetii* were detected on both aortic and mitral valve tissue using real-time PCR according to the Laboratory Response Network protocol from the Centers for Disease Control and Prevention. Each DNA extract was tested

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**Fig. 1.** Histological findings from aortic valve tissue. (a) Haematoxylin and eosin stain, magnification ×40. Microscopic aortic valve tissue demonstrating a combination of inflammatory infiltrate (distal portion, arrow) and nodular fibrocalcific degeneration (superior region, arrow). (b) Superior region of Fig. 1(a) (×100 magnification) demonstrating fibrocalcific degeneration. (c) Distal region of Fig. 1(a) (×100 magnification) demonstrating inflammatory infiltrate. (d) Warthin–Starry stain, magnification ×600. Clumps of silver-staining coccobacilli, bacteria consistent with *C. burnetii* infection.
against three *C. burnetii* DNA targets (*COX1*, *COX2* and *COX6*) using the human RNase P gene as the internal control (Table 2). All *C. burnetii* targets were positive from the aortic and mitral valves.

In the setting of strongly positive phase I and II Q fever serology and the detection of *C. burnetii* PCR products from cardiac valve tissue, a diagnosis of Q fever endocarditis was made, despite the history of appropriate Q fever screening and Q-Vax vaccination. Treatment was commenced with hydroxychloroquine 200 mg three-times daily and doxycycline 100 mg twice-daily. At a routine 3-month follow-up, he remained clinically well with good valvular function, normal inflammatory markers and resolution

**Table 1.** List of cross-reactive serology results in the patient with confirmed Q fever endocarditis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Assay</th>
<th>Results</th>
</tr>
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| *Coxiella burnetii* | Immunofluorescence, in-house assay, Australian Rickettsial Reference Laboratory | Phase 1  
IgA detected (titre > 3200)  
IgM not detected (titre < 25)  
IgG detected (titre > 3200)  
Total detected (titre > 3200)  
Phase 2  
IgA detected (titre > 3200)  
IgM not detected (titre < 25)  
IgG detected (titre > 3200)  
Total detected (titre > 3200) |
| *Legionella micdadei* | Immunofluorescence, in-house assay, Victorian Infectious Diseases Reference Laboratory | Total detected (titre 4096) |
| *Mycoplasma pneumoniae* | EIA, Fujirebio Serodia Myco II  
EIA, Bio-Rad Platelia | Total antibody titre 80  
IgM negative |
| *Bartonella henselae* | Immunofluorescence, in-house assay, Institute of Clinical Pathology and Medical Research | Total antibody titre 1024 |
| Spotted fever group *Rickettsia* | Immunofluorescence, in-house assay, Australian Rickettsial Reference Laboratory | *Rickettsia australis* detected (titre= 512)  
*Rickettsia honei* detected (titre= 512)  
*Rickettsia conori* detected (titre= 512)  
*Rickettsia sibirica* detected (titre= 512)  
*Rickettsia rickettsii* detected (titre= 512)  
*Rickettsia akari* detected (titre= 512) |
| Typhus group *Rickettsia* | Immunofluorescence, in-house assay, Australian Rickettsial Reference Laboratory | *Rickettsia prowazekii* detected (titre= 128)  
*Rickettsia typhi* detected (titre= 128) |
| Scrub typhus group *Rickettsia* | Immunofluorescence, in-house assay, Australian Rickettsial Reference Laboratory | *Rickettsia tsutsugamushi* serotype  
Gilliam: not detected (titre < 128)  
Karp: not detected (titre < 128)  
Kato: not detected (titre < 128)  
*ND* |
of previously troubling night sweats. A follow-up positron emission tomography scan demonstrated no metabolic activity of the known dilated aortic arch or prosthetic aortic or mitral valve.

**Discussion**

This is a rare case suggestive of Q fever vaccine failure resulting in chronic Q fever and endocarditis. This case highlights the need to consider the diagnosis even in patients with an appropriate vaccination history, particularly if they have pre-existing valvular heart disease. While there was a long period between vaccination and diagnosis with endocarditis, there was clinical suggestion of Q fever infection within a year post-vaccination, suggesting early vaccine failure. Furthermore, the case demonstrates problems with cross-reactive serology in the setting of extensive epidemiological risk factors, and highlights the value of organism-specific PCR in establishing a diagnosis of infective endocarditis.

*C. burnetii* is a highly virulent, obligately intracellular, Gram-negative bacterium and the causative organism of Q fever. It causes acute illness and/or chronic disease such as endocarditis and other endovascular infections, chronic fatigue syndrome, chronic granulomatous hepatitis or osteomyelitis (Maurin & Raoult, 1999). Q fever endocarditis is rare. In a large series of 111 consecutive cases of Q fever in Australia, most presentations were of acute illness; endocarditis occurred in only one patient (Spelman, 1982). Nonetheless, *C. burnetii* causes approximately 5% of endocarditis cases in some regions (Fournier et al., 1996).

Q-Vax is a monovalent vaccine developed by the Commonwealth Serum Laboratories (CSL) from formaldehyde-inactivated purified phase I *C. burnetii* whole cells and has been available in Australia since 1989 (Parker et al., 2006). Q fever vaccination in high-risk groups such as abattoir workers has been responsible for a large reduction in Q fever notifications, especially since a national funded programme commenced in 2002 in Australia (Gidding et al., 2003). Vaccine-induced immunity takes approximately 13 days to develop, and vaccination more than 10 days after exposure offers no protection (Gilroy et al., 2001).

There is a large body of evidence of vaccine efficacy. In 1984, 924 abattoir workers were given Q-Vax, with no evidence of Q fever at 18 months post-vaccination (Marmion et al., 1984). This was supported by a review of 4000 patients trialled with Q-Vax; all eight cases of endocarditis developed prior to the expected timing of vaccine-mediated immunity, suggesting that vaccination occurred during the incubation period of natural Q fever infection (Marmion et al., 1990). Furthermore, a 5 year follow-up study demonstrated that only two patients from a cohort of 2553 vaccinated patients developed breakthrough Q fever infection. These cases occurred within 2 days of vaccination, again suggesting natural Q fever infection rather than true vaccine failure (Ackland et al., 1994). The importance of Q-Vax is further underlined by a meta-analysis demonstrating a global effectiveness of 97% (up to 100% if patients with disease onset within 15 days of vaccination were excluded) (Gefenaite et al., 2011). This further confirms that disease seldom exists if vaccination occurs correctly. Routine post-vaccine serology is not recommended due to resultant long-term cell-mediated immunity and successful vaccine efficacy (Ackland et al., 1994; Marmion et al., 1990; Gefenaite et al., 2011). Moreover, most cases of Q fever endocarditis occur in patients with previous valvulopathies (Houpikian & Raoult, 2005), highlighting the importance of vaccination and increased vigilance in those with underlying valvular pathology. Endovascular infections of aneurysms and vascular grafts have also been reported and may be underappreciated (Maurin & Raoult, 1999).

The diagnosis of Q fever endocarditis is notoriously difficult. Commonly vegetations are not evident on echocardiogram, and intra-operative suspicion by the surgeon is usually rare. Histopathological changes are often mistaken for degenerative changes due to extensive fibrosis, small vegetations and limited inflammation (Lepidi et al., 2003). In our case, the clinical suspicion of endocarditis only arose intra-operatively. Furthermore, the diagnosis of Q fever endocarditis is commonly complicated by serology cross-reactivity. For example, 50% of cases have positive *Bartonella* spp. serology (La Scola & Raoult, 1996). Our patient had positive serology for *Bartonella henselae*, *L. micdadei* and *M. pneumoniae*, consistent with other reports in the literature (La Scola & Raoult, 1996; Musso & Raoult, 1997). Cross-adsortion assays may clarify the correct diagnosis; however, these are generally limited by the need for large amounts of antigen (Stein & Raoult, 1992). The availability of primers derived from genes specific to *C. burnetii* has allowed simple and reliable detection of DNA by PCR from tissue, more sensitive than standard culture or broad-range PCR techniques (Stein & Raoult, 1992).

We consider that this case represents a unique occurrence of Q fever endocarditis in an at-risk individual, following vaccine failure. There is evidence of negative *C. burnetii* pre-vaccination testing and appropriate vaccination. There was no report of disease onset within the acute period following vaccination, suggesting that it did not occur during the natural incubation period; however, this remains a less likely differential. Intraoperative suspicion, histological staining and organism-specific PCR enabled identification of Q fever endocarditis. Predisposing valvular dysfunction

### Table 2. *C. burnetii* real-time PCR results from mitral and aortic valve tissue

<table>
<thead>
<tr>
<th>Specimen</th>
<th>COX1</th>
<th>COX2</th>
<th>COX6</th>
<th>RNase P</th>
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<tbody>
<tr>
<td>Mitral valve</td>
<td>21.98</td>
<td>24.35</td>
<td>20.80</td>
<td>23.84</td>
</tr>
<tr>
<td>Aortic valve</td>
<td>21.27</td>
<td>20.15</td>
<td>20.21</td>
<td>24.34</td>
</tr>
<tr>
<td>Positive control</td>
<td>34.32</td>
<td>33.14</td>
<td>34.27</td>
<td>NA</td>
</tr>
</tbody>
</table>

Numbers are indicative of cycle threshold values. NA, Not applicable.
and appropriate epidemiology should increase suspicion for Q fever endocarditis, regardless of a history of vaccination. Due to well-documented problems with serological cross-reactivity in Q fever, and the inability to yield a diagnosis using broad-range 16S rRNA gene PCR, we encourage the use of organism-specific PCR in culture-negative endocarditis to aid diagnosis. Extra vigilance in routine valve replacements by surgeons, histopathologists, microbiologists and physicians alike in the setting of appropriate epidemiology may increase Q fever endocarditis detection rates.

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References


