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## Answer to Photo Quiz: Q Fever Endocarditis

(See page 3139 in this issue [[doi:10.1128/JCM.02563-12](https://doi.org/10.1128/JCM.02563-12)] for photo quiz case presentation.)

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The diagnosis was Q fever endocarditis of a native aortic valve. Figure 1 in the photo quiz shows *Coxiella burnetii* as numerous small Gram-variable (Gram-negative as well as Gram-positive) coccobacilli. The diagnosis of *C. burnetii* endocarditis was established by positive PCR results for *C. burnetii* DNA from the cardiac valve tissue specimen and peripheral blood. A 16S rRNA sequence analysis of the same tissue specimen yielded a PCR product of approximately 900 bp and a sequence that was 99.9% identical to *C. burnetii* (GenBank accession number [HM208383.1](https://www.ncbi.nlm.nih.gov/nuccore/HM208383.1)) over 775 bp. The serological test results were consistent with chronic *C. burnetii* infection, with titers for *C. burnetii*-specific antibodies as follows: a complement fixing antibody assay (CFA) titer of >10,240 (using *C. burnetii* phase II antigen; Virion/Serion GmbH, Wurzburg, Germany) and microscopic immunofluorescent antibody (IFA) assay titers of 2,048 for IgM anti-phase I, 65,536 for IgG anti-phase I, 4,096 for IgM anti-phase II, and 65,536 for IgG anti-phase II (Focus Diagnostics, Cypress, CA, USA). Long-term antibiotic treatment with doxycycline and hydroxychloroquine was initiated. Piperacillin-tazobactam was added to the regimen for *Bacteroides fragilis* bacteremia and for the uncultured Gram-variable organisms of unknown identity at that time. Piperacillin-tazobactam treatment was stopped after the 16S rRNA sequence results from the cardiac valve tissue were available. Since the blood cultures collected prior to antibiotic treatment were negative after prolonged incubation and 16S rRNA sequence analysis revealed a single sequence only, dual-pathogen endocarditis was ruled out (1). *B. fragilis* bacteremia was considered to have resulted from bacterial translocation from the gut during visceral ischemia due to the severe forward failure. The patient recovered and was discharged to the referring hospital. The patient was well at a postoperative visit with the cardiac surgeon 6 weeks after discharge.

*C. burnetii* is an important cause of culture-negative endocarditis. Q fever presents as either acute or chronic Q fever. In acute Q fever, a nonspecific self-limiting flu-like illness is common. In contrast, chronic Q fever is severe and includes endocarditis, vasculitis of the large vessels, or infection of vascular prosthesis (2). Q fever occurs as sporadic cases as well as in outbreaks, associated mainly with environmental contamination and/or contact with infected animals or animal products. From 2007 to 2010, the largest known Q fever outbreak occurred in the province of Brabant in the Netherlands. The outbreak subsided after a number of measures, including vaccination of livestock.

Diagnosis of Q fever is based on clinical findings in combination with microbiological investigations. Since culture of *C. burnetii* by use of cell culture is difficult and requires biosafety level 3 containment facilities, this type of culture is not performed in routine clinical microbiology laboratories. Laboratory diagnosis includes serology and PCR performed on peripheral blood or in-

fectured tissue specimens. Serology is the most frequently used diagnostic method. Classically, IFA assay is used, but enzyme-linked immunosorbent assay (ELISA) and CFA assay are also performed in Dutch laboratories. In acute Q fever, phase II-specific antibodies as determined by IFA assay predominate, while in chronic Q fever, phase I antibodies predominate. However, this classical picture has been challenged by the investigations from the Netherlands outbreak. In acute Q fever, phase II antibodies may still be present up to 1 year later as determined by follow-up, and phase I-specific antibody titers of <1,024 may occur in chronic Q fever (2). Serological follow-up is warranted during and/or after treatment of acute and chronic Q fever to monitor the response to treatment. *C. burnetii* is classified as a Gram-negative bacterium on the basis of cell wall composition and phylogenetic studies. Two case reports of Q fever endocarditis describe the presence of Gram-negative bacilli in Gram stains of cardiac vegetations (3, 4). Variation of the Gram stain protocol used and/or variation of the cell wall composition may have caused the Gram-variable appearance of *C. burnetii* in our patient (5). Regarding the latter, *C. burnetii* exists as small cell variants (SCV) or large cell variants (LCV) (6). Peptidoglycan layers in SCV are thicker than those from LCV and may therefore be harder to decolorize (7). Nevertheless, most reports and textbooks do not describe Gram stain for diagnosis of Q fever endocarditis, and if they do, they report that no bacteria could be visualized. In some cases, light microscopy revealed positivity by indirect immunofluorescent staining of cardiac tissues (8). Therefore, the sensitivity of the Gram stain for visualization of *C. burnetii* is possibly very low. In addition to poor staining properties, failure to observe *C. burnetii* in Gram-stained smears of cardiac valve tissue may be due to the small size of the bacterium, previous antibiotic treatment, and selection of unaffected parts of the valve for microscopy (sampling error). The presented case reconfirms that *C. burnetii* may be visualized in Gram stain preparations of infected cardiac valve tissue.

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