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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) 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 bacterium, previous antibiotic treatment, and selection of unaffected 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.

REFERENCES


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