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A Case of Q Fever Prosthetic Joint Infection and Description of an Assay for Detection of *Coxiella burnetii*

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We present the first published case of *Coxiella burnetii* prosthetic joint infection. Diagnosis was established with PCR and culture of periprosthetic tissue and synovial fluid (and serology). A novel PCR assay is described herein. Q fever should be considered in patients with prosthetic joint infection without an identified pathogen.

Joint replacement is a life-enhancing procedure performed on hundreds of thousands of patients in the United States each year. While prosthetic joint infection (PJI) occurs in only approximately 2% of patients following knee arthroplasty (1), it is a devastating complication. Accurate microbiologic diagnosis of PJI is important for directing appropriate management. There is no identified pathogen in approximately 7% of PJI cases, frequently as a result of antecedent antimicrobial therapy, but occasionally due to an inability to detect a pathogen by standard laboratory methods (2). *Coxiella burnetii* is not an organism that would traditionally be considered in a case of culture-negative PJI. We describe here the first published case of PJI caused by *C. burnetii*.

The causative agent of the zoonosis Q fever, *C. burnetii*, is a small, obligate intracellular Gram-negative bacterium. Acute and chronic types of Q fever are classically diagnosed based on compatible clinical illnesses, supported by serologic results (3). Isolation of *C. burnetii* in cell culture is laborious, requires specially trained staff, and poses a safety risk to staff (4, 5), confining *C. burnetii* culture to a small number of research and public health laboratories and limiting medical practitioners' access to this technique for diagnostics.

Q fever PCR offers a safe alternative to culture for direct detection of *C. burnetii* in clinical specimens. The majority of PCR assays for *C. burnetii* have targeted the insertion sequence element IS*1111* (6–10). As a mobile genetic element, there is the potential for IS*1111* to move from one organism type to another, theoretically resulting in loss of specificity (11). The diagnosis of *C. burnetii* PJI in the case reported here was made using a novel real-time PCR assay (described below) targeting the single-copy housekeeping gene target, shikimate dehydrogenase (*aroE*) of *C. burnetii*.

CASE REPORT

A 56-year-old male presented to our institution with new increasing right knee pain and swelling around a previously revised total knee arthroplasty (TKA).

He first developed right knee pain after patellectomy for a comminuted patellar fracture at 27 years of age. He underwent repeated arthroscopies without improvement; right TKA was performed at age 44. This was complicated by early postoperative deep vein thrombosis (DVT). He developed increasing right knee pain, swelling, and stiffness approximately 12 weeks after arthroplasty. Subcutaneous corticosteroid and lidocaine injections were administered around the branches of the saphenous nerve on a monthly basis for presumed neuropathic pain.

At the age of 53, he presented to our facility with a 5-day history of general malaise, mild nonproductive cough, and chills, along with right knee effusion and right posterior knee and calf pain in the context of an acute DVT. He had an erythrocyte sedimentation rate (ESR) of 57 mm/h (from 7 mm/h previously), C-reactive protein (CRP) of 39 mg/liter (from <3 mg/liter previously), and a normal peripheral leukocyte count. A roentgenogram of the right knee showed a well-seated prosthesis, but an indium-labeled leukocyte scan suggested soft tissue inflammation and reactive bone formation, possibly representing loosening of the arthroplasty. Joint space aspiration yielded 5 ml of slightly turbid, straw-colored synovial fluid, with 6,136 leukocytes/μl (71% neutrophils, 21% monocytes). Cultures of the aspirate were negative, and he did not receive antimicrobial therapy.

He continued to have pain and receive monthly subcutaneous injections. A painful hemorrhagic Baker's cyst developed and was drained. Approximately 8 months later, the pain and swelling again increased and the popliteal cyst was drained at another institution. Cultures of the popliteal cyst fluid were negative, and he was empirically treated with oral levofloxacin for 2 weeks. He returned to our facility shortly after this; inflammatory markers were again elevated. Indium-labeled leukocyte scan demonstrated findings consistent with popliteal and patellofemoral soft tissue infection around the prosthesis, along with loosening of the components, without definite evidence for osteomyelitis. Aspiration of the knee demonstrated 9,256 leukocytes/μl (69% neutrophils, 16% monocytes). Aerobic and anaerobic bacterial cultures were negative. Culture-negative PJI was diagnosed. He was not treated with additional antibiotics at the time; revision was delayed by patient preference.

Repeat joint aspiration 6 months later demonstrated 5,304 leu-

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kocytes/μl (24% neutrophils, 45% monocytes), with negative cultures while off any antibiotics. His ESR was 17 mm/h, and CRP was 3.3 mg/liter. Resection arthroplasty was performed. Operative examination showed necrosis and inflamed synovium around the implant; periprosthetic histologic examination of the tissue revealed acute and chronic inflammation. Bacterial, mycobacterial, and fungal cultures from four operative specimens were negative. All components were resected and a vancomycin- and tobramycin-impregnated polymethylmethacrylate spacer was implanted. He completed a 6-week treatment course with empirical parenteral vancomycin and oral levofloxacin.

Two months after resection, he underwent removal of the cement spacer and arthroplasty reimplantation using vancomycin- and tobramycin-impregnated polymethylmethacrylate. Periprosthetic histopathology revealed chronic inflammation. At the time of surgery, ESR was 20 mm/h and CRP was 14.5 mg/liter. Antibiotics were discontinued after operative cultures were finalized as negative. He had relatively good function of the new prosthesis but continued to have local injections for neuralgia in the right infrapatellar region.

At the age of 56, he developed new, increasing right knee pain and swelling without fevers or systemic symptoms. Inflammatory markers again were elevated, with an ESR of 40 mm/h and a CRP of 35 mg/liter. Aspiration of the joint fluid demonstrated 5,960 leukocytes/μl (76% neutrophils, 19% monocytes), with negative cultures. A roentgenogram of the right TKA did not reveal periprosthetic lucency.

He underwent right knee irrigation and debridement, with polyethylene exchange 2 months later. Periprosthetic histologic examination of the tissue revealed chronic inflammation. The preoperative ESR was 56 mm/h and CRP was 78.5 mg/liter. Bacterial, fungal, and mycobacterial cultures were negative. A work-up for culture-negative PJI revealed a strongly positive Q fever serology, with phase I IgG of 1:4,096 and phase II IgG of 1:2,048; IgM for both phases I and II was negative. Immunohistochemistry using *C. burnetii* antibodies was negative on the right knee soft tissue. PCR for *C. burnetii aroE* (described below) performed on periprosthetic tissues and synovial fluid in the Mayo Clinic Clinical Microbiology Laboratory was positive. Periprosthetic tissue was further tested at the Université de la Méditerranée, Marseille, France, where PCR for the 16S rRNA gene was negative, but PCR for *C. burnetii* IS*1111* repetitive elements was positive (7); the specimen was inoculated onto human endothelial cell (ECV 304) shell vials and grew doxycycline-susceptible *C. burnetii* (3).

The patient was started on oral ciprofloxacin and doxycycline. Transesophageal echocardiogram was negative for vegetations. Repeat serology performed 2 weeks after starting treatment demonstrated a rise in phase II IgG to 1:8,192 with unchanged phase I antibodies. ESR declined to 14 mm/h and CRP declined to 3.4 mg/liter after 1 month of treatment. He developed Achilles tendon pain, so ciprofloxacin was changed to trimethoprim-sulfamethoxazole, which was subsequently discontinued due to hyperkalemia. Oral doxycycline was continued, and hydroxychloroquine was added subsequently. He has had no evidence of relapsed infection 11 months after starting therapy.

MATERIALS AND METHODS

Mayo Clinic Real-Time PCR assay. (i) Assay design. Primers and probes were designed to amplify and detect a region of the shikimate dehydrogenase gene (*aroE*) using LightCycler Probe Design, version 2.0 (Roche Applied Science), and Beacon Designer 8 (Premier Biosoft International, Palo Alto, CA) software (Table 1).

TABLE 1 Primer and probe sequences for the Mayo Clinic real-time PCR assay

Name	*Coxiella burnetii aroE* primer or probe sequence
Primers	
QfevF	5′ TTTGCCTTTCAAAGAAGAAGCAT 3′
QfevR	5′ ATTGTGATTGCGAGTGAGAT 3′
Probes	
Qfevfl	5′ AGAAGCCAATGAAGCCCACG FITC 3′
QfeviLC610	5′ Red610 GCCAGCGCTTTGCAATTTCGAGAAGAC PO4 3′
QfevRTfl	5′ CACTGAACCAATGTCTGCTCTGATTTG FITC 3′
QfevRTiLC670	5′ Red670 CAAGATTTGTTGTAGCTGTTTCAGCTA TGTCCAT PO4 3′

(ii) Positive control and recovery template. To create the positive control, a fragment of *aroE* was amplified using the PCR primers and inserted into *Escherichia coli* using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Transformed organisms were plated, incubated, and distinguished on imMedia Kan Blue (Invitrogen, Carlsbad, CA) agar plates after overnight incubation. Conventional PCR was performed on the transformed isolate, and the product of this reaction was run on a 2% agarose gel (e-gel; Invitrogen, Carlsbad, CA) to assess size and confirm that a single product was amplified. Additionally, the amplified PCR product was bidirectionally sequenced.

The transformed organisms were grown overnight to an approximate turbidity of 4 McFarland in imKan Liquid (Invitrogen, Carlsbad, CA) broth and pelleted, and the supernatant was removed. Plasmid DNA was isolated using the Roche High Pure Plasmid isolation kit (Roche Applied Science, Indianapolis, IN). The final eluate was quantified using a Nanodrop 2000 microsample spectrophotometer (Thermo Scientific, Wilmington, DE). The concentrated plasmid was diluted in 1× Tris-EDTA buffer (Sigma-Aldrich, St. Louis, MO) and stored at −20°C.

A recovery template was created by synthetically generating the target region of *aroE* and replacing the assay probe region with an alternate nucleotide sequence homologous to the alternative probe set QfevRTfl and QfevRTiLC670 (Table 1) (GenScript, Piscataway, NJ).

(iii) PCR. Fifteen μl of the PCR master mix containing 1× FastStart DNA Master HybProbe (*Taq* DNA polymerase, PCR buffer, deoxyribonucleoside triphosphate with dUTP substituted for dTTP, 1 mM $MgCl_2$), 3 mM (additional) $MgCl_2$, 0.5 μM forward and reverse primers, and 0.3 μM each of the hybridization probes was added to each 20-μl LightCycler reaction cuvette. Extracted nucleic acid (5 μl) was added to each cuvette. Positive and negative extraction controls, consisting of 100 copies of the plasmid target suspended in 50% S.T.A.R. buffer (Roche Applied Science, Indianapolis, IN) and 1,000 cells/μl of *E. coli* ATCC 25922, were extracted and assayed with the clinical specimens. Amplification and detection of recovery template, by melting-curve analysis, was used to assess the presence of postextraction PCR inhibitors.

The thermocycler program was the following: 95°C for 10 min; 45 amplification cycles of 10 s at 95°C, 15 s at 55°C (single acquisition), and 15 s at 72°C; melting-curve analysis of 0 s at 95°C, 20 s at 59°C, 20 s at 45°C (ramp rate of 0.2°C/s), and 0 s at 85°C (ramp rate of 0.2°C/s and continuous acquisition), and finally cooling for 30 s at 40°C.

(iv) Assay validation panel. A panel of 20 extracted specimens containing *C. burnetii* DNA derived from 7 human specimens and 13 cheese samples was kindly provided by Didier Raoult, Université de la Méditerranée, Marseille, France (Table 2).

PJI specimen processing and testing. Three knee tissues and a synovial fluid aspirate were subjected to processing and nucleic acid extraction

TABLE 2 Assay validation panel

Specimen source	*aroE* PCR crossing point/melting temp (°C)	IS*1111* PCR crossing point
Abscess	34.53/65.84	29.4
Abscess	33.61/65.75	26
Heart valve	32.52/65.80	27
Heart valve	29.83/65.27	24
Heart valve	31.96/65.47	26
Blood	35.93/65.98	31
Aortic biopsy	28.54/65.13	22
Cheese 102810	34.01/66.15	30
Cheese 102884	35.74/66.33	31
Cheese 102915	35.83/66.40	29
Cheese 101862	36.22/65.69	30.5
Cheese 102759	35.88/66.02	30.5
Cheese 102763	36.26/65.75	30.5
Cheese 102764	34.06/65.94	30
Cheese 102768	34.69/66.01	31
Cheese 102779	34.64/66.24	30
Cheese 102781	33.69/65.72	30
Cheese 102782	34.09/65.86	30
Cheese 102799	32.29/65.98	29
Cheese 102801	35.55/66.29	30

as previously described (12). Briefly, portions of each tissue, measuring approximately 0.1 by 0.1 by 0.5 cm, were placed into a sterile 1.5-ml microcentrifuge tube containing 400 μl of 1× Tris-EDTA buffer (Fisher Scientific, Waltham, MA) and 50 μl of 10% sodium dodecyl sulfate (Sigma-Aldrich, St. Louis, MO). Four-hundred microliters of the synovial fluid was transferred into a sterile 1.5-ml microcentrifuge tube along with 50 μl of 10% sodium dodecyl sulfate. One-hundred microliters (100 μl) of proteinase K (Roche Applied Science, Indianapolis, IN) was added, and the suspensions were placed onto an Eppendorf thermomixer (Hauppauge, NY) overnight at 55°C at 400 rpm. Nucleic acid was extracted from 200 μl of digested material on the Roche MagNA Pure Compact instrument using the Total Nucleic Acid kit (Roche Molecular Diagnostics, Indianapolis, IN), yielding 100 μl of eluate.

Analytical sensitivity, specificity and cross-reactivity. Analytical sensitivity was assessed by assaying a series of dilutions of genomic DNA in 1× Tris-EDTA buffer from the *C. burnetii* Nine Mile strain. In addition, limit-of-detection studies in sample matrix were performed. Briefly, six serial, 10-fold dilutions of genomic DNA were prepared in digested tissue material. Each dilution was extracted in triplicate and assayed in duplicate. The limit of detection was estimated by probit linear regression with corresponding 95% confidence intervals using StatsDirect statistical analysis software, version 2.7.7 (StatsDirect Ltd., Cheshire, England). To determine analytical specificity, the predicted amplified product and primer and probe sequences were subjected to BLAST queries using the National Center for Biotechnology Information (NCBI) genomic database (http://www.ncbi.nlm.nih.gov). Cross-reactivity studies were performed using a panel of 35 organisms (Table 3).

RESULTS

Mayo Clinic real-time PCR assay. (i) Analytical sensitivity, specificity, and cross-reactivity. The analytical sensitivity of the *aroE* PCR assay was 2 *C. burnetii* targets/μl. The limit of the detection in fresh tissue was estimated by probit analysis to be 10 (95% confidence interval of 2 to 45) targets/μl. Amplified product, primer, and probe sequences were subjected to NCBI database searches using BLAST software. No significant homology was noted. None of the 35 organisms included in the cross-reactivity panel was detected.

TABLE 3 Cross-reactivity panel

Organism	Source	*aroE* PCR result
Afipia felis	ATCC 53690	Negative
Acinetobacter lwoffii	Mayo isolate	Negative
Babesia microti	Mayo isolate	Negative
Bacillus anthracis	Mayo isolate	Negative
Bartonella henselae	ATCC 49882D-5	Negative
Bartonella quintana	ATCC 51694	Negative
Borrelia burgdorferi	ATCC 35210	Negative
Brucella species	Mayo isolate	Negative
Candida albicans	Mayo isolate	Negative
Corynebacterium species	ATCC 49676	Negative
Epstein-Barr virus	Mayo isolate	Negative
Enterococcus faecium	ATCC 35667	Negative
Enterococcus faecalis	ATCC 19433	Negative
Escherichia coli	ATCC 25922	Negative
Francisella tularensis	Mayo isolate	Negative
Human DNA	MRC-5 line	Negative
Klebsiella pneumoniae	ATCC 700603	Negative
Listeria monocytogenes	ATCC 15313	Negative
Legionella pneumoniae	ATCC 33152	Negative
Tatlockia (*Legionella*) *micdadei*	ATCC	Negative
Mycobacterium avium	ATCC 700898	Negative
Mycobacterium tuberculosis	ATCC 35838	Negative
Mycoplasma pneumoniae	ATCC 29085	Negative
Neisseria meningitidis	ATCC 13077	Negative
Pasteurella multocida	CAP D-20-77	Negative
Pseudomonas aeruginosa	ATCC 27853	Negative
Staphylococcus aureus	ATCC 29923	Negative
Staphylococcus epidermidis	ATCC 14990	Negative
Streptococcus pneumoniae	ATCC 49619	Negative
Streptococcus pyogenes	ATCC 19615	Negative
Streptococcus bovis group	CAP D-16-88	Negative
Streptococcus anginosus group	Mayo isolate	Negative
Streptococcus salivarius group	Mayo isolate	Negative
Streptococcus mitis group	Mayo isolate	Negative
Yersinia pestis	Mayo isolate	Negative

(ii) Assay validation panel. The *aroE* assay detected all specimens in the validation panel (Table 2).

PJI testing. Three out of four specimens (two tissues and the aspirate) were positive for *C. burnetii*, producing melting peaks identical to that of the positive control. The PCR amplicons from the positive patient specimens were bidirectionally sequenced; the sequences were identical to *aroE* from *C. burnetii* Dugway 5J108-11 (NC_009727.1). The PCR recovery template in the negative specimen was detected, indicating the absence of PCR inhibitors.

DISCUSSION

Q fever is a worldwide zoonosis caused by the intracellular bacterium *C. burnetii*. Ungulates, such as goats, sheep, and cattle, are considered the primary source for human infection (3). Acute and chronic forms are differentiated by temporality of illness, disease presentation, and serologic findings (13). Patients with acute Q fever are often asymptomatic, with the most common manifestations of symptomatic disease being pneumonia, hepatitis, or isolated fever. In contrast, the majority of patients with chronic Q fever have endocarditis. Less common syndromes include vascular graft or aneurysm infection or chronic infection following pregnancy (14).

Osteoarticular infection is an uncommon presentation, present in only 7 of 313 reported Q fever cases (2%) during a large serologic study extending over 14 years in France (14). A number of cases of osteoarticular infection have previously been reported (15–21). Osteomyelitis is the most common manifestation of osteoarticular infection, with histopathology showing noncaseating granulomas (15, 16, 18, 19) and lymphoplasmacytic infiltrates (19). Tenosynovitis has been reported, with pathology showing granulomas (17). Vertebral spondylodiscitis and paravertebral abscess have been reported (17, 21). Diagnosis is based on PCR detection of *C. burnetii* from bone or tissue (17–19), growth of *C. burnetii* in cell culture (16, 17), or compatible illness in conjunction with positive serology (15, 20, 21). There has been a single prior case report of *C. burnetii* native hip joint infection following arthroplasty resection (20). To our knowledge, PJI due to *C. burnetii* has never been reported.

PJI is caused by Gram-positive cocci in approximately two-thirds of cases. Polymicrobial, anaerobic, and Gram-negative infections occur less frequently (22). Approximately 7% of PJI cases are culture negative. Newer techniques, such as prosthesis sonication (23) and broad-range PCR, may increase the percentage of cases with defined microbiology (24). Chronic PJI frequently involves biofilm formation, but it is unclear whether *C. burnetii* is capable of biofilm formation.

Our patient presented with pain, knee effusion, and elevated synovial leukocyte count 8 years after primary arthroplasty. While there was no microbiologic evidence to confirm that *C. burnetii* was the causative pathogen at that time, his later diagnosis supported this. His relapse 3 years later, despite 6 weeks of levofloxacin and a two-stage arthroplasty replacement, suggests that the treatment of Q fever PJI should be similar in duration to that of other serious chronic Q fever illnesses, with combination treatment given for at least 18 months. Our patient lived in an urban area and had no known exposure that would obviously place him at risk for Q fever. However, *C. burnetii* may be underrecognized; for example, its DNA can be detected by PCR in over 90% of bulk milk samples in the United States, highlighting an ongoing reservoir of *C. burnetii* (25). Clinicians should consider *C. burnetii* in the microbiologic differential diagnosis of culture-negative PJI; the assay described herein should be useful in making this diagnosis.

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