Detection of *Legionella bozeman*, a New Cause of Septic Arthritis, by PCR Followed by Specific Culture

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***Legionella bozeman* is a rare isolate in clinical specimens. We describe a case of joint infection due to *L. bozeman* in an immunocompromised patient with dermatomyositis. Without the use of PCR screening or culture on specialized medium, the organism would not have been detected.**

CASE REPORT

A 71-year-old woman with amyopathic dermatomyositis presented to the department of rheumatology with septic arthritis of the left knee. Her medical history included several admissions to the department during 2011, where she underwent a comprehensive diagnostic workup for dermatomyositis with underlying malignancy.

During the investigation, three skin biopsy specimens were obtained, one close to the interphalangeal joint of her right thumb, one from the anterior aspect of the chest, and one from the right thigh. All of the skin lesions appeared as erythematous-to-violaceous papules and plaques.

Unfortunately, the biopsy specimen from the thumb was complicated by septic arthritis due to *Streptococcus agalactiae*, followed by *Staphylococcus aureus* wound and bloodstream infections. However, the patient gradually recovered and the diagnostic program found no signs of malignancy. During the entire diagnostic workup, the patient had normocytic, normochromic anemia with hemoglobin levels of 5.0 to 7.0 mmol/liter.

The patient was diagnosed with amyopathic dermatomyositis and started on immunosuppressive treatment with methotrexate (20 mg weekly), prednisolone (20 mg daily), and hydroxychloroquine (250 mg daily) and then discharged to a rehabilitation stay at a rheumatologic hospital. At discharge, both her white blood cell (WBC) count and her C-reactive protein (CRP) level were within normal ranges.

During this stay, the patient developed intermittent pain in her left thigh, with a concomitant rise in the CRP level to 24 mg/liter. During the next months, the pain in the thigh worsened and she experienced malaise and weight loss. Examination at our hospital revealed a swelling of her left knee. Ultrasound showed a large intracavitary joint fluid effusion. Five milliliters of purulent aspirate was obtained from the left knee joint cavity (WBC count not available). Gram staining with carbol fuchsin as a counterstain showed numerous neutrophilic granulocytes but no bacteria or fungi. No crystals were detected. Cultures grown under standard conditions were negative (agar plates with 10% horse blood and thioglycolate broth [Statens Serum Institute, SSI Diagnostica, Hilleroed, Denmark] incubated for 48 h at 35°C with 5% CO₂). Blood samples showed anemia (5.4 mmol/liter), leukocytosis (WBC count of 13.9×10^9 /liter with the following differential analysis: neutrophils, 12.4×10^9 /liter; eosinophils, 0.0×10^9 /liter; basophils, 0.0×10^9 /liter; lymphocytes, 0.7×10^9 /liter; monocytes, $0.8 \times$

10^9 /liter), and an increased CRP level (54 mg/liter). X-rays of the left knee and chest were unremarkable. Blood and urine cultures were negative.

Methotrexate treatment was withdrawn, and intravenous cefuroxime (1,500 mg three times daily) was started, but after 12 days, she was still not clinically improving and positron emission tomography-computed tomography (PET-CT) was done. It showed strong fluorodeoxyglucose uptake in the left knee joint (Fig. 1A). There was no abnormal skeletal or pulmonary activity (Fig. 1B).

Subsequently, the patient had worsening of pain, 60 ml of gray-white aspirate was obtained from the left knee cavity, and 2 ml of methylprednisolone acetate (40 mg/ml) was injected. This second aspirate (WBC count, 80×10^9 /liter) was also found to lack bacteria, crystals, or fungi by standard methods. But since the suspicion of bacterial infection was strong, a broad-range 16S PCR assay was done, resulting in the finding of *Legionella bozeman* with a 99.48% match. The PCR was performed using the Fast MicroSEQ 500 16S rDNA PCR kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol, and the region amplified and sequenced was the first 500 bp of the 16S rRNA gene. We now knew what we were looking for, and specific culture of the same (second) joint fluid sample on Oxoid modified Wadovsky Yee (MWY) plates gave, after 3 days of incubation in 5% CO₂, growth of >100 small greyish colonies that were indistinguishable from colonies of *Legionella pneumophila* and were identified as *L. bozeman* by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry with equipment from Shimadzu/Saramis (Shimadzu Corporation, Kyoto, Japan, and later bioMérieux). The species identification was confirmed at the *Legionella* Reference Laboratory, Statens Serum Institute, Copenhagen, Denmark, by MALDI-TOF (Bruker Daltonics microflex LT) and sequencing of the *mip* gene. To verify that this finding was due to infection rather than contamination, a third aspiration of joint fluid (after 1 week of treatment with ce-

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FIG 1 PET-CT of the patient just before the second aspiration of fluid from the left knee, where 60 ml of joint fluid was obtained. (A) Lateral view of the left knee. (B) Whole-body view with no lung activity.

furoxime) was performed. This time, the 16S PCR assay was negative but culture on MWY plates gave growth of a few colonies of *L. bozeman* and treatment with ciprofloxacin (500 mg twice daily) was initiated. After the shift in therapy, the thigh pain gradually improved and the CRP and hemoglobin levels normalized. Antibiotic treatment was continued for 3 weeks.

We performed a search for the origin of the infection with water samples taken from the patient's home, the rehabilitation hospital, and our department; however, *L. bozeman* could not be detected. Tap water analyses were performed at the *Legionella* Reference Laboratory, Statens Serum Institute, Copenhagen, Denmark, and so were analyses of specific antibodies of the patient's serum. Serum samples collected 3 and 6 weeks after the first joint puncture were analyzed for antibodies to *L. pneumophila* by enzyme-linked immunosorbent assay (ELISA) as previously described (4). For this study, antigens (lipopolysaccharide extracts) from the *L. bozeman* isolate from the patient and from a *Legionella anisa* environmental isolate were also prepared for ELISA. No antibodies to *L. pneumophila* were detected (optical density [OD] values of <0.010), but high levels of IgM and IgG antibodies against *L. bozeman* (OD values for both samples were around 3.0 for both IgM and IgG) were detected and some expected "cross"-reaction with the closely related species *L. anisa* (OD values of around 1.5 both for IgM and IgG) were also seen. Ten randomly selected serum samples from patients without known recent legionellosis were also analyzed for IgM and IgG antibodies to *L. bozeman* and *L. anisa*, and the median OD values were, respectively, 0.161, 0.106, 0.542, and 0.021. It must be noted that the ELISAs for the two non-*L. pneumophila* species were experimental and not validated routine tests.

In the present case of suspected infectious arthritis, repeated standard cultures of synovial fluid were negative. An additional

broad-range 16S PCR assay of the joint fluid proved to be an essential diagnostic aid in identifying *L. bozeman*, which was subsequently confirmed by specific cultures. The diagnosis was further supported by high levels of IgM and IgG antibodies against *L. bozeman* in serum. To our knowledge, *L. bozeman* has not previously been reported as a cause of septic arthritis.

L. bozeman is a rare cause of legionellosis, which preferentially affects immunocompromised hosts, leading to cavitary lung lesions (10, 14, 15). The mortality rate of *L. bozeman* pneumonia has been reported to be up to 40% (14). Diagnosing *L. bozeman* is difficult since both the urinary antigen test for *Legionella*, targeted against *L. pneumophila* serogroup 1, and serological kits fail to detect *L. bozeman* (9, 15). Furthermore, even microbial cultures can be false negative on cefamandole-containing media because *L. bozeman* does not grow there (11).

Legionella spp. may also cause primary extrapulmonary disease, and septic arthritis has been reported. Four cases of septic arthritis (2, 3, 7, 12), two cases of reactive arthritis (1, 13), and one case of a prosthetic joint infection (6) have been reported in the past. These pathogens were *L. pneumophila* (four cases), *Legionella longbeachae* (one case), *Legionella dumoffii* (one case), and *Legionella micdadei* in a prosthetic joint infection. The median age of the patients was 64 years, and 62.5% were immunocompromised.

A broad-range 16S PCR assay has previously been applied to bone and joint infections (5). One conclusion was that this method should be used for patients highly suspected of having culture-negative infections. Also, the 16S PCR assay has been used successfully to detect *Legionella* spp. in joint infections (6, 7).

In the second joint fluid aspirate, which was analyzed with the 16S PCR assay, *L. bozeman* was detected and confirmed with specific culture. The subsequent joint aspiration (9 days later) failed to find the organism by 16S PCR assay, but a few colonies were detected by specific culture, emphasizing that the two methods should be combined. The broad-range 16S PCR assay has

drawbacks, such as a risk of contamination and false-positive results (5, 8).

Furthermore, *L. bozemanii* is not susceptible to cefuroxime, which was used as the first-choice antibiotic in this case, and in general, *Legionella* spp. are often resistant to standard empirical treatments, which shows the importance of considering rare pathogens when an infection does not resolve following standard therapy.

In conclusion, this report demonstrates that *L. bozemanii* can cause infectious arthritis and cannot be detected by using standard cultures. However, the etiology can be determined by using a 16S PCR assay and sequencing, followed by culture under specific conditions. The infection can be successfully treated by using ciprofloxacin.

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