**Desulfovibrio desulfuricans** Bacteremia in a Dog

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*Desulfovibrio desulfuricans* was isolated from the blood of a dog presenting with fever, anorexia, and rear limb stiffness. The isolate was identified by 16S rRNA gene amplification and sequencing.

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**CASE REPORT**

A two-and-half-year-old male Labrador retriever presented with anorexia and rear limb stiffness. Immunoserologic tests for *Ehrlichia* and *Brucella canis* were negative. Lyme serology, however, were positive and consistent with prior vaccination and/or natural exposure. The dog was started on oral cefadroxil but after 3 days developed a fever of 105°F and a tense abdomen. The antibiotic was changed to doxycycline, 200 mg daily, for the next 3 days. A blood sample for bacterial culture was taken 12 h after the doxycycline was stopped. Small, rounded, pinpoint, smooth colonies appeared on chocolate agar plates under anaerobic conditions after 5 days' incubation. Doxycycline therapy was resumed for 4 weeks, and the dog had a gradual improvement with no recurrence of symptoms.

Sulfate-reducing *Desulfovibrio* spp. are ubiquitous in nature (3). They have been isolated from most environmental sources, such as sewage, industrial effluents, and most types of water and soil. They are also constituents of the normal anaerobic floras of the human and animal digestive tracts (7–10). However, occasionally they can become opportunistic pathogens (5, 9). Reports of clinical cases involving *Desulfovibrio* species are rare in part because they are present in small numbers in the gut. Moreover, they are difficult to isolate in culture, as they are obligately anaerobic, very slow growing, fastidious bacteria. However, new molecular genetics methods, such as PCR and sequencing of 16S ribosomal DNA (rDNA), have contributed to the detection and identification of many slow-growing, uncommon, or unculturable pathogens. Indeed, at least six clinical cases of *Desulfovibrio* spp. have been confirmed in humans in recent years by 16S rDNA analysis. *Desulfovibrio* species have been isolated from appendiceal tissue as well as peritoneal fluid from patients with acute perforating appendicitis (1). In addition, cases of bacteremia, bacteriuria, liver abscesses, and septicemia caused by *Desulfovibrio fairfieldensis* have been described (11, 13, 17). The bacterium was associated with fever, whereas the bacteriuria was associated with meningococcalitis. *Desulfovibrio desulfuricans* has been reported to be the cause of a case of gastrointestinal disorder (4) and bacteremia with episodes of fever, chills, and profuse perspiration (15). Several groups of researchers have reported *Desulfovibrio*-like organisms, based on 16S rDNA sequence similarity, associated with proliferative bowel diseases from ferrets, pigs, and hamsters (2, 6, 12, 14). We describe a case of bacteremia associated with high fever, stiff limbs, and tense abdomen caused by *D. desulfuricans* in a dog from Wisconsin.

Our isolate grew as tiny, pinpoint, round colonies on anaerobic chocolate agar plates after 5 days of incubation at 37°C from dog blood culture bottles. Microscopic examination of the Gram-stained smears showed gram-negative, slightly curved bacteria. Electron microscopic studies revealed a single polar flagellum on curved to rod-shaped cells (Fig. 1). Other forms, such as bullet- or cigar-shaped cells, were also observed. The isolate was characterized by amplifying (Secpexg, pld) analyzing its 16S rRNA gene. The template DNA for the 16S rDNA PCR was prepared from several colonies suspended in 200 μl of sterile water, boiled for 10 min, and centrifuged briefly to pellet the cell debris. The supernatant was collected and used directly as the template. Universal prokaryotic primers, FD1 (5′-AGA GGT TGA TCC TGG CTC AG-3′) and RD1 (5′-AAG GAG GTG ATC CAG CC-3′), were used to amplify a 1,540-bp segment from the 16S rRNA gene (18). The PCR was performed using the GeneAmp PCR reagent kit and AmpliTaq DNA polymerase (Perkin-Elmer, Branchburg, N.J.). A 100-μl PCR mixture contained 10 μl of 10× PCR buffer; 1.4 mM MgCl₂; 200 μM concentrations of dATP, dCTP, dGTP, and dTTP; 2.5 U of *Taq* polymerase; 20 pmol each of FD1 and RD1 primers; and 5 μl of the template DNA. The single PCR product was column purified and then directly sequenced by cycle sequencing using the Thermo Sequenase kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, Piscataway, N.J.) and Cy5-labeled nested primers (18). The sequencing reaction products were electrophoresed in a 5% sequencing gel for 12 h in an ALF Express DNA sequencer (Amersham Pharmacia Biotech). The 1,476-bp 16S rDNA sequence was compared to all bacterial sequences available in the GenBank database by using the Blast 2.0 program (National Center for Biotechnology Institute) and showed 99% homology with the sequence from *D. desulfuricans* ATCC 27774 (GenBank accession no. 34113). Based on this information, a desulfoviridin test was performed, which was positive (16).

To our knowledge, this is the first case report of bacteremia caused by *D. desulfuricans* in a dog. Based on the clinical history, it probably originated in the gastrointestinal tract. *Desulfovibrio* species are heterogeneous in nature and have diverged greatly in their 16S rRNA sequences (3). For example, phylogenetically *D. desulfuricans* strain ATCC 27774 is more closely related to *Desulfomonas pigra* strain ATCC 29098 than to *D. desulfuricans* Norway 4 and *D. desulfuricans* EI Agheila Z (3). Interestingly, *D. fairfieldensis*, a newly named isolate from three human cases, has been placed between the strains *D. desulfuricans* ATCC 27774 and *Desulfomonas pigra* ATCC 29098 (17). The 16S rDNA sequences of our dog isolate had only 96% identity with the *D. fairfieldensis* sequence (GenBank accession no. U42221), indicating that it was not related to the
human isolate (11, 13, 17). More clinical isolates of Desulfovibrio spp. from humans and other mammals will determine the diversity of pathogenic strains within this genus.

Isolation and identification of fastidious, anaerobic bacteria—like Desulfovibrio—by traditional microbiological methods will always be difficult. They do not grow well in broth medium. Even if they do, their growth is rather difficult to monitor because of the lack of any visible change in the optical density of the growth medium for a number of days. On a solid medium, it may take 5 to 7 days for a visible colony to appear. Not surprisingly, most reports of identification of Desulfovibrio spp. from clinical specimens rely more on molecular identification. Analysis of the 16S rRNA gene sequences has helped to detect and distinguish Desulfovibrio spp. from other pathogens, such as Campylobacter and Lawsonia-like organisms (2, 6).

Based on our experience, it appears that 16S rRNA gene analysis is useful in detecting slow-growing anaerobes, like D. desulfuricans.

Nucleotide sequence accession number. The 16S rRNA gene sequence of the isolate has been deposited into GenBank (accession number AF098671).

REFERENCES