ENDOCARDITIS CAUSED BY *ROCHALIMAEA HENSELAE*

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A case of community-acquired, culture-negative, infective endocarditis was diagnosed in a 57-year-old construction worker. Small, pleomorphic gram-negative rods were seen in Brown-Hopps tissue gram stains and Warthin-Starry silver stains. The organism was identified as Rochalimaea henselae by polymerase chain reaction amplification and sequencing of the 16S rDNA gene sequence. This is the first report of infective endocarditis caused by R henselae. HUM PATHOL 24:1140–1141. This is a US government work. There are no restrictions on its use.

The diagnostic hallmarks of infectious endocarditis (IE) are valve failure and culture of an infectious agent from peripheral blood. However, sterile blood cultures are reported in 2.5% to 13% of patients with IE. Several factors may increase the incidence of sterile cultures: (1) cultures are taken toward the end of a chronic course (longer than 3 months), (2) intervention of uremia in a chronic course, (3) slow growth or inappropriate growth conditions for fastidious organisms, (4) prior administration of antibiotics, (5) organisms that are obligate intracellular parasites, and (6) infection with a previously unidentified organism with unique growth requirements.[1]

In culture-negative IE cases identification of the etiologic agent depends on histopathology. Often, morphologic features of *Staphylococcus*, *Streptococcus*, or *Neisseria* organisms are characteristic. It is much more difficult to identify the genus of gram-negative rods based on histopathology. In such culture-negative cases polymerase chain reaction (PCR) technology may be the only means of identification.

CASE REPORT

A 59-year-old black male construction worker had fatigue, decreased energy, fever, chills, and a recent onset of a productive cough. There was swelling of his lower extremities for several weeks, shortness of breath, and alcohol abuse, but the patient denied use of intravenous drugs or exposure to animals.

Physical examination revealed severe edema of the lower extremities, hyperthermia (39.6°C), bibasilar lung rales, +S3 gallop, and a holosystolic heart murmur grade 4/6. Chest x-ray films showed cardiomegaly.

Laboratory findings were sodium 133 mEq/L, calcium 8.2 mg/dL, lactate dehydrogenase 280 U/L, white blood cell count 8,300/mm^3, granulocytes 79.5%, lymphocytes 15.9%, and monocytes 4.6%. The patient had negative serologies for the human immunodeficiency virus.

Several blood cultures were drawn and the patient was started on vancomycin and gentamicin. Echocardiographic examination revealed changes consistent with aortic and tricuspid vegetation with severe regurgitation. All cultures of blood, urine, and sputum were reported as negative for pathogens.

The patient underwent valve replacement surgery. Unusual-appearing vegetation was noted on the free edges and on both sides of the aortic and tricuspid valves. Cultures of aortic and tricuspid valve tissue were negative for bacteria and fungi. Sections of aortic and tricuspid valves stained with hematoxylin-eosin showed a dense eosinophilic fibrinoid matrix along the margin of the valve extending back over the leaflets (Fig 1). The matrix appeared granular, but infectious agents were not observed. A diagnosis of infective endocarditis was made, but the etiologic agent remained unknown. The case was forwarded to the Armed Forces Institute of Pathology for evaluation. Following surgery the patient's temperature returned to 37°C and antibiotic therapy was changed to ampicillin plus sulbactam. The lower extremity edema resolved and the patient was discharged 34 days after admission.

Pathologic Findings

On the hematoxylin-eosin–stained sections the organisms were not distinct and did not stain well. Brown-Hopp's tissue gram stains and Warthin-Starry silver stains clearly revealed small, pleomorphic, gram-negative rods in collagen surrounding vessels and in heaping masses on the leaflets. Silver impregnation enlarged the organisms and revealed beaded, filamentous, and branching bacteria. Because the morphologic features of the bacteria and the granular appearance of the vegetation were distinctly different from the usual causes of IE, we isolated DNA from the paraffin-embedded tissue for amplification with universal bacterial primers.

Four paraffin-embedded, 6 μm-thick tissue sections were placed in a sterile 1.5-mL Eppendorf tube and DNA was extracted using the method of Wright and Manos.[2] Universal primers were adapted from Relman et al.[3]

Ten microliters of DNA extract was sufficient for the amplification of eubacterial 16S rRNA gene. The reaction mix contained 20 pmol of each primer and recommended amounts of amplification reagents supplied in the Applied Biosystems PCR/sequencing kit (Applied Biosystems, Foster City, CA). A negative control consisted of reaction mixture without DNA template; a positive control consisted of pUC plasmid with a 600 base pair fragment supplied with the kit. Amplified DNA samples (20 μL) were electrophoresed in a 1% agarose gel and bands were detected by ethidium bromide staining.

Sequencing (373A DNA sequencer and the Taq DyeDeoxy Terminator Cycle Sequencing Kit, Applied Biosystems) of the PCR products gave a 176 base pair sequence with p11E plus p13B primers and an approximately 480 base pair sequence with p93E plus p13B primer (Fig 2). Sequences were analyzed and ambiguous base calls were resolved by aligning sequence data generated with forward and reverse primers. DNA was not amplified from the template-deficient tube or from tissue not containing bacteria. The final sequence data were compared with all 16S rRNA sequences from the GenBank (Los Alamos, NM)/European Molecular Biology Laboratory (Heidelberg, Germany). The 176 base pair fragment exactly matched the sequence reported by Relman et al for the ba-

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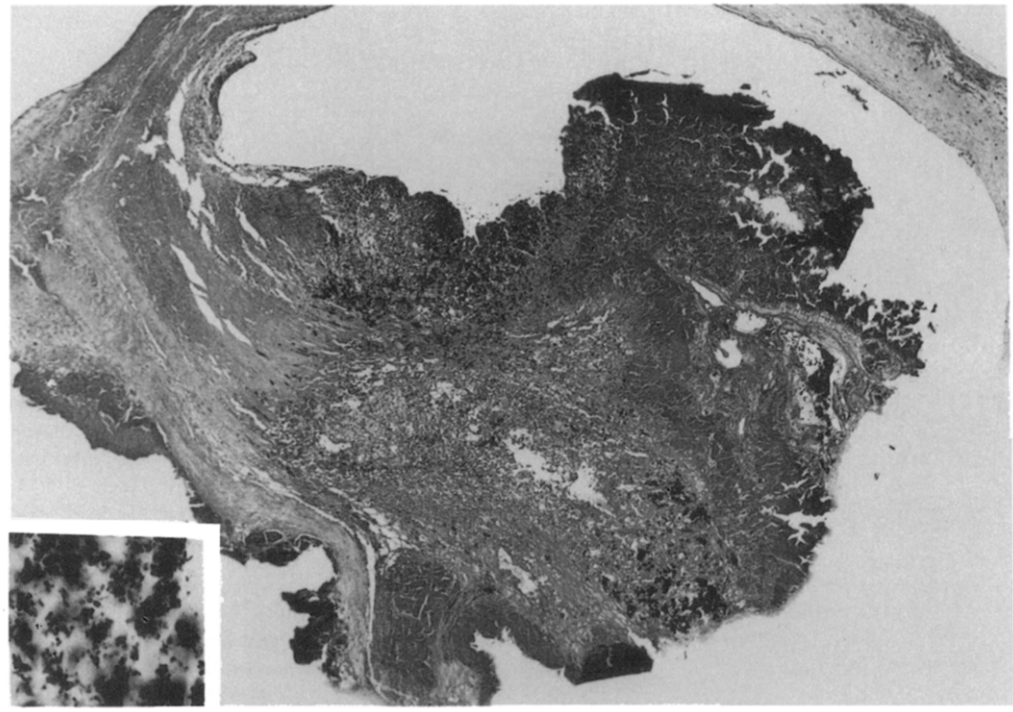

FIGURE 1. A hematoxylin-eosin–stained aortic heart valve with extensive vegetation present on both sides of the valve. (Magnification ×30.) (Inset) A Warthin-Starry–stained section of the aortic heart valve showing small rod-shaped bacteria in clumps and as individual organisms. (Magnification ×430.)

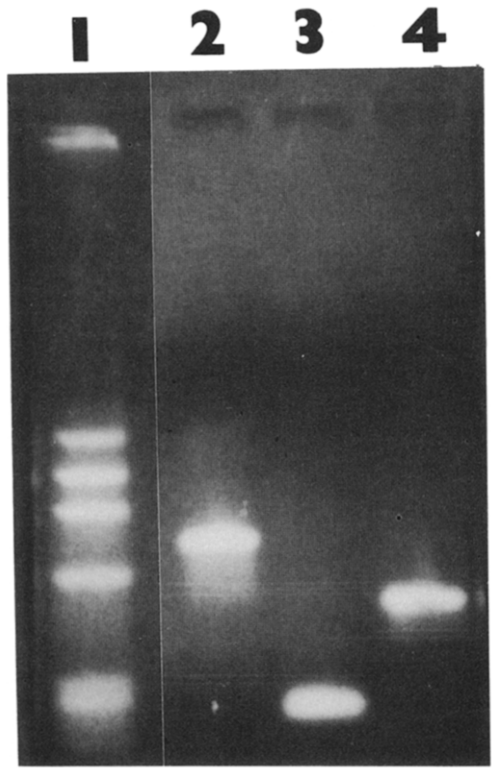

FIGURE 2. Agarose gel electrophoresis of PCR-amplified 16S rDNA fragments from paraffin-embedded heart valve tissue. Broad-range procaryotic primers were used for PCR amplification of bacterial DNA from paraffin-embedded tissue. Lane 1 contains an Hae III digest of Phi X (molecular weight markers). Lane 2 contains a PCR-positive control supplied with PCR amplification kits. Lane 3 is a heart valve DNA extract amplified with p11E and p13B universal primers. The PCR product is approximately 180 base pairs. Lane 4 is a heart valve DNA extract amplified with p93E and p13B universal primers. The PCR product is approximately 500 base pairs.

cillary angiomatosis agent[3] more recently named *Rochalimaea henselae*.[4]

DISCUSSION

This report describes the first patient with native valve IE due to *R henselae*. This organism usually causes bacillary angiomatosis[5,6] or peliosis hepatica[7] in human immunodeficiency virus patients and was cultured from the blood of bacillary angiomatosis (human immunodeficiency virus) patients, allogeneic transplant recipients, and noncompromised patients.[8] Another patient with *R henselae* infection was reported to have a thickened aortic valve based on echocardiogram findings, but a diagnosis of IE was not established.[5] We believe many patients with *Rochalimaea* sp infection are misdiagnosed because of its small size and poor staining characteristics, and because the organism is not readily grown in culture. This first report of endocarditis due to *R henselae* expands the spectrum of disease caused by this newly emerging pathogen.

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