SALINAS-MELÉNDEZ, J.A.; R. TAMEZ-GONZÁLEZ; O. WELSH-LOZANO & H.A. BARRERA-SALDAÑA. Detection of *Borrelia burgdorferi* DNA in human skin biopsies and dog synovial fluid by the Polymerase Chain Reaction. *Rev. Lat.-Amer. Microbiol.* 37:7-10, 1995.

To determine the value of the PCR assay as a diagnostic tool for Lyme disease, a specific PCR assay targeting within the genes encoding the flagellin, the major outer surface protein (OspA) or 16S rRNA11[15], was used for the detection of *Borrelia burgdorferi* in skin biopsy specimens from human patients and from synovial fluid from a dog clinically suspected of having Lyme disease[1].

MATERIAL AND METHODS

Patients: Puncture biopsy specimens were obtained from two patients attending the Department of Dermatology, University Hospital with a history and skin manifestations suggestive of Lyme disease.

As negative controls, DNA from human subjects with no history of clinical disease and from *Escherichia coli* bacteria were included. Biopsy specimens and synovial fluid were stored frozen (-70 °C) until the DNA was extracted for PCR.

B. burgdorferi isolate: The *B. burgdorferi* strains B31 DNA used as a positive control was kindly donated by Dr. Ulf B. Göbel from the Institut für Medizinische Mikrobiologie und Hygiene of the Freiburg University of Germany.

Bacterial strains. Strain B31 (ATCC 35210), the prototype strain, was isolated from *Ixodes dammini*[3] and is used as a bacterin for the prevention of Lyme disease in dogs (Fort Dodge Laboratories, USA).

DNA extraction procedures. Biopsy samples and synovial fluid. The DNA from the skin biopsy samples and synovial fluid were extracted as previously described[11,15]. The DNA from the *B. burgdorferi* strain (bacterin) was isolated as described previously[14,17].

Amplification of Borrelia burgdorferi DNA. The PCR was performed essentially as described previously[1,17]. Oligonucleotides (BBTP1 and BBRT1) were kindly donated by Dr. Göbel. Amplification of *B. burgdorferi*-specific target sequences (16S rRNA gene sequences) was carried out in 100 µl reaction mixture containing 250 ng of DNA from the patient specimens or 1ng of DNA from the *B. burgdorferi* strain B31 or the bacterin, 8 µM (each) deoxynucleotide triphosphates (i.e., dATP, dTTP, dGTP and dCTP), 2.5 µM of primer sets and 10 U of the TaqDNA polymerase. The reaction was overlaid with mineral oil (100 µl) to prevent evaporation and was subjected to 35 cycles of amplification by using an automated DNA Thermal Cycler (MJ Research). Each cycle involved heating to 94 °C for 2 min (DNA denaturation), cooling to 50 °C for 2 min (primer annealing), and again heating to 70 °C for 2 min (primer extension). Determination of the PCR amplification products was performed by gel electrophoresis on a 2% agarose gel or 8% polyacrylamide with ethydium bromide staining. Aliquots (5 µl) of amplified fragments, 244 bp in length, were separated on 2% agarose gels and stained with ethydium bromide.

RESULTS

Amplification primers BBUTP1 and BBURT1 generated a 244 fragment representing part of the variable region V4 of the *B. burgdorferi* 16S rRNA gene.

The specificity of the primer set for the detection of *B. burgdorferi* strain was determined by the amplification of an American (Strain B31) and a bacterin strain. By using this primer set, a specific fragment of 244 bp was generated in both strains (Fig.1).

PCR for B. burgdorferi in patients samples. It was possible to obtain fresh skin biopsy from 2 patients with acrodermatitis. A single PCR with the primer set on the DNA extracted from these skin samples and on the DNA extracted from synovial fluid from a dog involved in this study is shown in Fig. 2 This PCR resulted in the specific amplification of a 244 bp fragment from the 2 patients (Fig. 2 lane B and C) and the dog (Fig. 2 lane D). No specific amplification was seen from the *E. coli* strain (Fig. 2 lane A).