JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1990, p. 566–572
0095-1137/90/030566-07$02.00/0

Vol. 28, No. 3

Detection of *Borrelia burgdorferi* Infection in *Ixodes dammini* Ticks with the Polymerase Chain Reaction†

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Received 11 August 1989/Accepted 16 November 1989

The polymerase chain reaction (PCR) was used to amplify DNA sequences of the etiologic agent of Lyme disease, *Borrelia burgdorferi*, and was applied to the detection of the spirochete in its tick vector. The target for PCR amplification was the OSP-A gene of strain B31; analysis of isolates from different geographical areas indicated that this gene could be used to identify most North American isolates. These methods were extended to the analysis of colony-derived and field-collected *Ixodes dammini*. OSP-A-specific sequences were identified in 15 of 15 colony-derived nymphal ticks that had fed previously on an infected animal; no such amplification products were detected in 8 control ticks. Segregated midgut tissues of field-collected adult and nymphal ticks from Nantucket Island, Mass., and the Crane Reserve, Ipswich, Mass., were examined by both direct fluorescent-antibody (DFA) staining and PCR. The DFA technique identified 16 infected ticks of 30 paired specimens; 15 of these specimens were positive by PCR. One specimen was positive by PCR that was DFA negative. Both live whole ticks and desiccated dead specimens were suitable for this analysis. Because only live ticks are suitable for DFA analysis, the use of PCR may extend the range of specimens that can be analyzed for the presence of the Lyme spirochete.

Lyme disease is a complex multisystem disorder caused by the tick-borne spirochete *Borrelia burgdorferi* (32). The deer tick, *Ixodes dammini*, is the primary vector in the northeastern United States, Wisconsin, and Minnesota (30). *I. pacificus* and *I. ricinus* are the main vectors in the western United States and Europe, respectively (16). The prevalence of infected ticks varies widely among geographic regions and is directly proportional to the number of reported cases of Lyme disease in a given location (31, 34). Screening tests for the presence of spirochetes in the arthropod vector currently consist of examination of midgut contents dissected from live ticks, using fluorescent antibodies or culture or both (2, 4). However, these methods cannot be applied to the analysis of dead specimens, which may constitute a substantial proportion of those specimens brought to physicians and veterinarians after tick bite. A test that could be applied to both living and dead ticks may be useful in predicting the likelihood of an exposed individual developing Lyme disease, as well as in determining the prevalence of infected ticks at a given collection site.

The recently described polymerase chain reaction (PCR) is a powerful technique that is especially useful for the detection of pathogens whose in vitro cultivation is difficult, lengthy, or unavailable (22, 24, 28). For example, PCR may be used to detect a single human immunodeficiency virus type 1-infected cell among 10^5 uninfected cells (23). Application of this technique to the detection of *B. burgdorferi*, whose slow growth properties and infrequent isolation from tissues have limited primary culture as a diagnostic procedure (6, 32), would be an important step in expanding the diagnostic repertoire for this important human pathogen. Accordingly, we used the PCR to detect *B. burgdorferi* DNA sequences in *I. dammini*. We compared its sensitivity and specificity with those of the direct fluorescent-antibody (DFA) procedure in detecting evidence of infection in both colony-derived and field-collected ticks.

MATERIALS AND METHODS

Sequences and strains. The target for PCR amplification was the gene encoding the OSP-A protein of strain B31. The complete nucleotide sequence of this gene has been determined recently (13). The nucleotide sequences for the OSP-A and OSP-B genes were kindly provided in advance of publication by Alan Barbour, University of Texas School of Medicine, San Antonio. Frozen stocks of isolates of *B. burgdorferi* were provided by one of us (S.W.B.) and John Anderson, Connecticut Agricultural Experiment Station, New Haven.

Source of infected ticks. Nymphal ticks were derived from a laboratory colony of *I. dammini*, originally collected on Great Island, West Yarmouth, Mass., and now in its second generation. They were infected with the JD-1 strain of *B. burgdorferi* as described previously (26).

Collection of ticks. Adult and nymphal *I. dammini* were collected during April and May 1989 from vegetation on the grounds of the University of Massachusetts Nantucket Field Station, on Nantucket Island, Mass., and on the Crane Reservation, Ipswich, Mass. Both are sites in which the agent of Lyme disease is intensely enzootic (21, 25). Ticks were kept at 15°C and 90% relative humidity with a 16-h photophase until processing.

Tick processing. Ticks were washed in distilled water, and their body contents were individually dissected into a drop of cold TE (10 mM Tris [pH 7.4], 0.5 mM EDTA) on a microscope slide. Half of the resulting intact material (primarily the gut diverticulae) was transferred into a 0.5-ml microcentrifuge tube containing 25 μl of TE, and tubes were placed on ice until frozen at −70°C. The remaining material was triturated on the slide and then dried at room temperature and fixed in acetone for 10 min. Forceps were dipped in

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† Contribution of the University of Massachusetts Nantucket Field Station.

acetone-alcohol and flamed after each dissection to prevent contamination by material from prior dissections.

DFA procedure. To detect infection in ticks, slides were directly stained with a fluorescein isothiocyanate-conjugated polyclonal rabbit anti-*B. burgdorferi* antibody, washed, mounted in buffered glycerol, and examined by epifluorescence microscopy at ×320 total magnification. A total of 100 random fields were examined before a tick was considered uninfected. The level of infection for each tick preparation was scored subjectively by the method of Lane and Burgdorfer (20).

SDS-PAGE. Pellets of *B. burgorferi* were washed in phosphate-buffered saline containing 5 mM $MgCl_2$, suspended in distilled water, analyzed for protein content, placed in incubation buffer to give a final protein concentration of 0.85 mg/ml, and processed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in an SE 600 vertical gel unit (Hoefer Scientific Instruments, San Francisco, Calif.) as described previously (5). The gel was stained with Coomassie brilliant blue R-250.

PCR amplification of OSP-A sequences from cultured isolates of *B. burgdorferi*. Oligonucleotide primers OSP-A1 and OSP-A2 were synthesized on an oligonucleotide synthesizer (Applied Biosystems, San Mateo, Calif.) (coupling efficiency, 99.5%), desalted on an oligonucleotide purification cartridge (Applied Biosystems), and used without further purification. For PCR amplification of stock strains of *B. burgdorferi*, genomic DNA was purified by proteinase K-SDS digestion followed by two phenol-chloroform extractions and ethanol precipitation. A 1-ng portion of genomic DNA was added to a 50-μl PCR reaction mixture containing 10 mM Tris hydrochloride (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.01% gelatin, 200 μM each deoxynucleoside triphosphate, and 50 pmol each of primers OSP-A1 and OSP-A2. All reactions were performed in a Perkin-Elmer–Cetus thermal cycler; components were denatured at 94°C for 30 s, annealed at 45°C for 45 s, and extended at 72°C for 2 min, for a total of 30 cycles. The amplification products were analyzed by electrophoresis in a 1.5% SeaKem (FMC Corp., Marine Colloids Div., Rockland, Maine) agarose gel followed by staining with ethidium bromide and UV transillumination.

To reduce risk of contamination of our experiments with PCR fragments, analysis of amplification products was carried out in a separate area from where PCR reaction tubes were set up; reagents to be used in sample setup were prepared in a separate room. Pipetting for sample and reagent preparation was carried out with positive displacement pipettes (SMI, Emeryville, Calif.) or a dedicated set of pipettors.

Restriction enzyme analysis of OSP-A amplification products. Amplification products from the OSP-A1/OSP-A2 primer pair were digested with *Sau*3a, *Alu*I, *Eco*RI, and *Pst*I (New England BioLabs, Beverly, Mass.) by using the manufacturer's recommendations. The digests were then analyzed by gel electrophoresis on a 3% Nusieve–1% SeaKem agarose gel (FMC Corp.), followed by ethidium bromide staining and UV transillumination.

Amplification of OSP-A sequences in *I. dammini* ticks. Whole nymphal ticks were placed in the bottom of a 0.5-ml microcentrifuge tube, covered with 20 μl of TE, and crushed with a pipette tip. The buffer became slightly cloudy when the contents of the tick had dispersed into the buffer. Alternatively, when dissected midgut specimens were analyzed, the specimen was dispersed in the buffer by vigorous pipetting. The tubes were then placed into a 100°C heating block for 10 min and immediately chilled on ice. A 5-μl amount of the supernatant was added to PCR buffer containing Tris hydrochloride (pH 8.3), 50 mM KCl, 1.75 mM $MgCl_2$, 0.01% gelatin, a 200 μM concentration of each deoxynucleoside triphosphate, and 50 pmol each of primers OSP-A2 and OSP-A4. Reaction components were subjected to 45 cycles of amplification, using the conditions described above. The amplification products were first analyzed by gel electrophoresis on a 3% Nusieve–1% SeaKem agarose gel and then slot blotted onto Hybond membranes (Amersham Corp., Arlington Heights, Ill.), using a slot blot manifold (American Bionetics, Hayward, Calif.). Membranes were cross-linked on a UV transilluminator for 5 min and prehybridized in 5× SSPE–5× Denhardt solution–0.5% SDS–100 μg of denatured salmon sperm DNA per ml at 55°C for 1 h. Filters were hybridized in the above solution with 2×10^6 cpm of ^{32}P-end-labeled OSP-A3 primer for 4 to 6 h. After hybridization, filters were washed twice for 5 min each at room temperature in 2× SSC–0.1% SDS and once at 55°C in 1× SSC–0.1% SDS for 30 min. Filters were then exposed to Kodak XAR-5 film between intensifying screens for times ranging from 1 h at room temperature to as long as 2 days at −70°C.

RESULTS

PCR amplification of OSP-A sequences from cultured isolates of *B. burgdorferi*. We first asked whether portions of the OSP-A gene are sufficiently conserved among isolates from different geographical areas to serve as reliable targets for PCR amplification. Genomic DNA was extracted from 10 cultured isolates obtained from various locations in the United States and Europe. Oligonucleotide primers OSP-A1 and OSP-A2 (shown schematically in Fig. 1) were used in PCR reactions containing 1 ng of genomic DNA (corresponding to approximately 10^5 genomes) and amplified for 30 cycles. Conditions for annealing were set to low stringency to allow for imperfect base pairing of primers with potentially divergent target sequences; this maneuver did not appreciably affect levels of background amplification. The amplification products were then subjected to gel electrophoresis in 1.5% agarose and visualized by staining with ethidium bromide (Fig. 2A).

The expected amplification product for strain B31 is 646 base pairs (bp) in length, encompassing the latter two-thirds of the OSP-A open reading frame (Fig. 1). Representative North American isolates from Connecticut (lane 1), two isolates from Minnesota (lanes 5 and 10), and two isolates from northern California (lanes 3 and 7), as well as an *I. ricinus* isolate from France (lane 8), gave amplification products of similar intensities that comigrated with that obtained from strain B31 (lane 2). In contrast, no detectable amplification products were observed from a German tick isolate (lane 6) or from unusual isolates from California (lane 4) and Millbrook, N.Y. (lane 9). PCR analysis of serial dilutions of cloned strain B31 and several of the other isolates showed comparable levels of sensitivity (data not shown).

PAGE analysis was performed on whole-cell lysates of the above isolates to determine the electrophoretic mobility of the OSP-A protein produced by each isolate (Fig. 2B). Such analysis has been used previously to differentiate between isolates; most isolates from North America demonstrate an OSP-A species of M_r 31,000 (9–11). A lysate prepared from strain B31 (lane 2) shows a predominant protein species migrating with an apparent molecular weight of 31,000; this

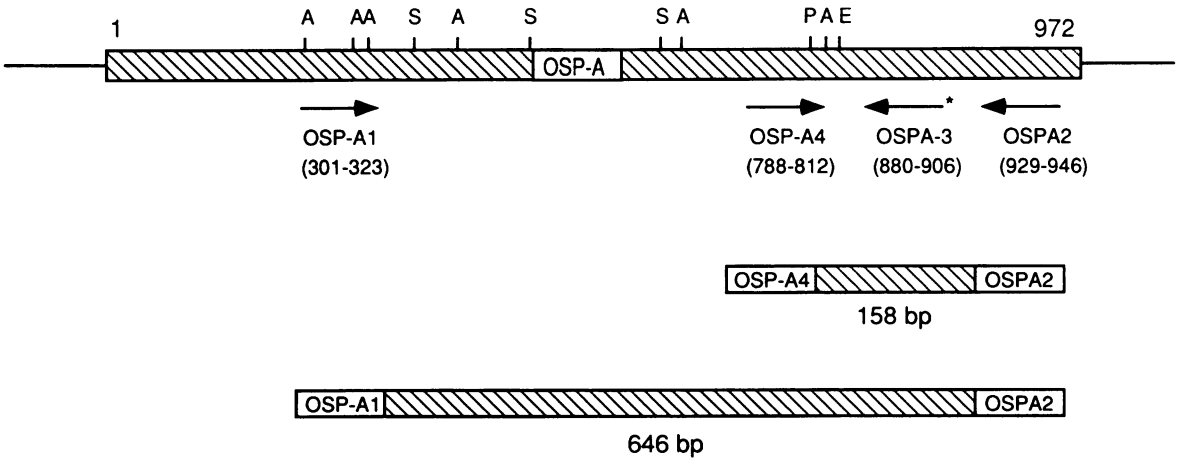

FIG. 1. Map of the OSP-A open reading frame and locations of primers used in PCR amplification. Shown is the restriction map of the OSP-A gene (hatched area) with four restriction enzymes: *Alu*I (A), *Sau*3A (S), *Pst*I (P), and *Eco*RI (E). The locations of the primers (arrows) used in the PCR reactions are indicated in parentheses and are numbered according to the first nucleotide of the OSP-A gene (13); the orientation of each primer is shown with the arrow pointing in the 5'-to-3' direction. Asterisk indicates the location of the ^{32}P-labeled oligonucleotide probe, OSP-A3. Below are shown the amplification products expected from the primer sets.

protein has been identified previously as OSP-A (9, 11). A protein of similar mobility was demonstrated in lysates from five North American isolates (lanes 1, 3, 5, 7, and 10) and one French isolate (lane 8). However, lysates prepared from the German isolate and the two atypical North American isolates demonstrated OSP-A proteins of slightly slower mobilities (lanes 4, 6, and 9), confirming previous studies (5, 15). Interestingly, the latter isolates also failed to produce a detectable amplification product by PCR (Fig. 2A, lanes 4, 6, and 9).

Nucleotide sequence conservation of the OSP-A gene among North American isolates. We were interested in whether significant nucleotide sequence divergence had occurred within the OSP-A gene among the isolates tested. Such information would be important in designing primer sets for use in PCR; restriction fragment length polymorphism analysis of the amplified segments might also lead to the development of strain-specific markers. Amplification products from six isolates were subjected to cleavage with restriction enzymes *Sau*3A and *Alu*I, which are predicted to recognize several sites within the 646-bp fragment (Fig. 1). The cleavage products were run on a 4% agarose gel and stained with ethidium bromide. The results of this analysis are shown in Fig. 3. *Alu*I cleavage yielded products of 231, 171, and 154 bp in length from the amplification products of the seven isolates shown in Fig. 2A. Digestion with *Sau*3A yielded predominant products of 335, 184, 58, and 62 bp. None of the amplification products from the seven isolates gave rise to a unique cleavage pattern; all fragments were of the size predicted by the sequence of the OSP-A gene for strain B31. Analysis with the restriction enzymes *Eco*RI and *Pst*I also yielded the same results for all seven isolates; *Eco*RI cleavage gave fragments of 500 and 146 bp, and *Pst*I cleavage yielded fragments of 491 and 155 bp (data not shown).

PCR detection of *B. burgdorferi* in colony-derived *I. dammini* ticks. We applied these techniques to the analysis of known infected and uninfected *I. dammini* ticks for the presence of *B. burgdorferi*-specific DNA sequences. Experimentally infected nymphal ticks were obtained by larval feeding on a *Peromyscus* mouse that had been tick inoculated with strain JD-1. This isolate had been shown previously to produce a 31-kilodalton OSP-A protein and to be reactive in our PCR-based assay (S. R. Telford III and D. H. Persing, unpublished observations). The interval between infected larval feeding and analysis was approximately 5 months. Control ticks that had fed on an uninfected animal were analyzed concurrently. The midguts of the ticks were dissected out; half of the midgut was triturated onto a glass slide for DFA analysis; the other half was placed into buffer for PCR. Numbers of spirochetes in each specimen detected by immunofluorescence were scored as follows: 1+, 1 to 2 spirochetes; 2+, 2 to 10 spirochetes; 3+, 10 to 100 spirochetes; 4+, 100 to 1,000 spirochetes. After the remaining midgut sections were boiled, 10 μl of the crude supernatant was used in a 50-μl PCR. Samples were then subjected to 45 cycles of amplification with primers OSP-A2 and OSP-A4, followed by slot blotting onto a nylon membrane. The amplification products were detected by hybridization to a ^{32}P-labeled oligonucleotide probe (OSP-A3), which corresponds to sequences flanked by the primers (Fig. 1).

Figure 4 shows that, whereas none of the eight control ticks gave rise to a *B. burgdorferi*-specific amplification product, all of the experimentally infected colony-derived ticks were positive. These results compare favorably with those obtained by DFA analysis of paired specimens; all 15 of the paired specimens were positive for spirochetes. A discrete 158-bp band was present on an ethidium-stained agarose gel for all 15 of the positive specimens (data not shown); no such species was present in the negative specimens or in the negative controls.

Detection of *B. burgdorferi* in field-collected ticks. We next analyzed field-derived material from Nantucket Island, Mass. Adult female ticks were collected by sweeping vegetation in April 1989. Midgut sections of the ticks were divided equally for DFA and PCR analysis, and numbers of spirochetes were scored by immunofluorescence staining as before. The remaining portions were subjected to 45 cycles of amplification, using primers OSP-A2 and OSP-A4 as above. Immunofluorescent-antibody studies and amplification reactions were conducted as double-blind experiments.

The amplification products were analyzed by gel electrophoresis and slot blot analysis as above. Figure 5A shows the gel electrophoresis results after amplification. The expected 158-bp amplification product was present in all of the

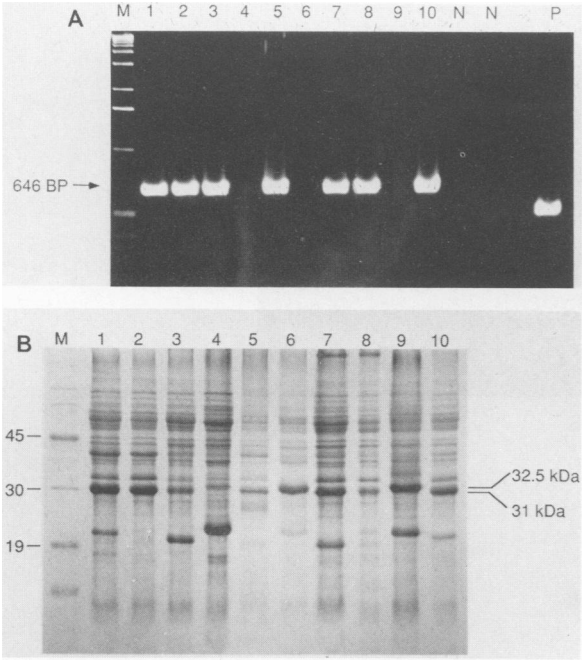

FIG. 2. PCR amplification and PAGE analysis of cultured isolates of *B. burgdorferi*. (A) Cultured isolates of *B. burgdorferi* from various locations were subjected to 30 cycles of PCR amplification of the OSP-A open reading frame, using primers OSP-A1 and OSP-A2 to produce a 646-bp amplification product. The fragment was subjected to gel electrophoresis in a 1.5% agarose gel and visualized by UV transillumination after being stained with ethidium bromide. Lane 1, Cloned strain N40 (Westchester County, New York); lane 2, cloned strain B31 (ATCC); lane 3, isolate SON 328 (northern California) (14); lane 4, isolate DN127 (northern California) (15); lane 5, Minnesota mouse isolate; lane 6, German tick isolate; lane 7, isolate from Lake County, California (14); lane 8, tick isolate from Foret de Pompet, France (1); lane 9, *I. dammini* isolate 25015 from Millbrook. N.Y. (5); lane 10, CD16, Minnesota tick isolate. (B) PAGE analysis of the cultured isolates was performed as described in the text. The heavily stained species at 31 kilodaltons (kDa) seen in lanes 1, 2, 3, 5, 7, 8, and 10 has been previously identified as the OSP-A protein (9, 11). A slightly larger, 32.5-kilodalton OSP-A-like species is seen in lanes 4, 6, and 9. The lane assignment is the same as in panel A. The sizes (10^3) of the molecular weight markers are indicated on the left.

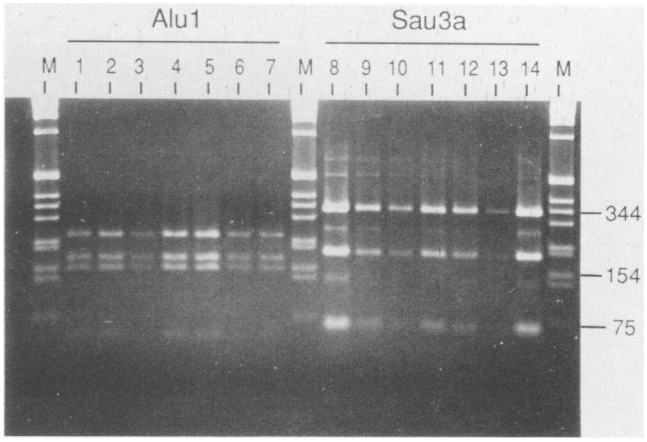

FIG. 3. Restriction enzyme digestion of the OSP-A amplification products. The 646-bp OSP-A amplification products derived from isolates shown in Fig. 2A were subjected to restriction enzyme cleavage with *Alu*I and *Sau*3a. *Alu*I cleavage yielded products of 231, 171, and 154 bp in length for all seven of the amplification products, as predicted by the sequence of the OSP-A gene of strain B31 (13). *Sau*3A cleavage yielded predominant products of 335, 184, 58, and 62 bp, also as predicted by the sequence. The lengths, in base pairs, of representative size markers are shown on the right.

paired specimens judged positive by immunofluorescence staining with the exception of sample 4. Slot blot analysis of the amplification products confirmed the results obtained by gel electrophoresis after a 1-h exposure (Fig. 5B); however, after an 18-h exposure, sample 5, which was negative by immunofluorescence staining, was repeatedly positive by PCR (data not shown). Positive specimens identified by PCR in this run corresponded to samples scored as 2+ (ca. 2 to 10 total spirochetes per midgut section) or 3+ (ca. 10 to 100 spirochetes) by immunofluorescence staining (Fig. 5A).

To exclude the possibility that division of an individual tick's body contents would reduce the sensitivity of our detection procedures, additional samples were prepared with whole nymphal ticks from a Crane Reserve, Mass., collection for which a rate of infection had been previously determined by immunofluorescence. Table 1 shows the results of this analysis. The midgut contents of 15 nymphal ticks were divided for comparative analysis as before. Seven of the 15 specimens were declared to be infected by DFA staining; the degree of infection was judged to be 1+ in one specimen, 2+ in one specimen, 3+ in 4 specimens, and 4+ in one specimen. All seven corresponding specimens were positive by PCR after both gel electrophoresis and slot blotting; no additional positives specimens were detected among those determined negative by DFA. We then performed DFA analysis and PCR amplification on whole ticks from the same collection. Live whole ticks were placed in the bottom of a 0.5-ml microcentrifuge tube, covered with 20 μl of distilled water containing 0.5% Tween 20, macerated with a pipette tip, boiled for 10 min, and placed on ice. A 10-μl portion of the supernatant was added directly to the PCR mixture, followed by amplification, gel electrophoresis, and slot blotting. In this analysis, 8 of 15 whole specimens

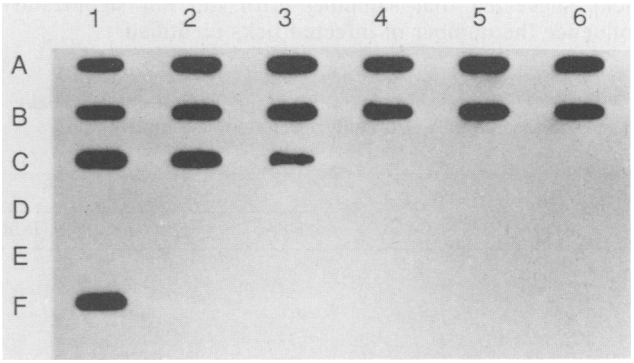

FIG. 4. PCR detection of *B. burgdorferi* in known positive colony-derived *I. dammini* ticks. Midgut contents of 15 ticks from a positive animal (slots A1 through C3) and 8 whole ticks from a negative animal (slots D1 through E2) were subjected to 45 cycles of amplification with primers OSP-A2 and OSP-A4 as described in the text. The amplification products were then slot blotted onto a nylon membrane and probed with ^{32}P-end-labeled OSP-A3 probe. The filter was then washed and exposed to film between intensifying screens for 18 h at room temperature. Slot F1 contains the positive control (strain N40 DNA); slots F2 and F3 contain negative controls (distilled water added to the reaction mixture).

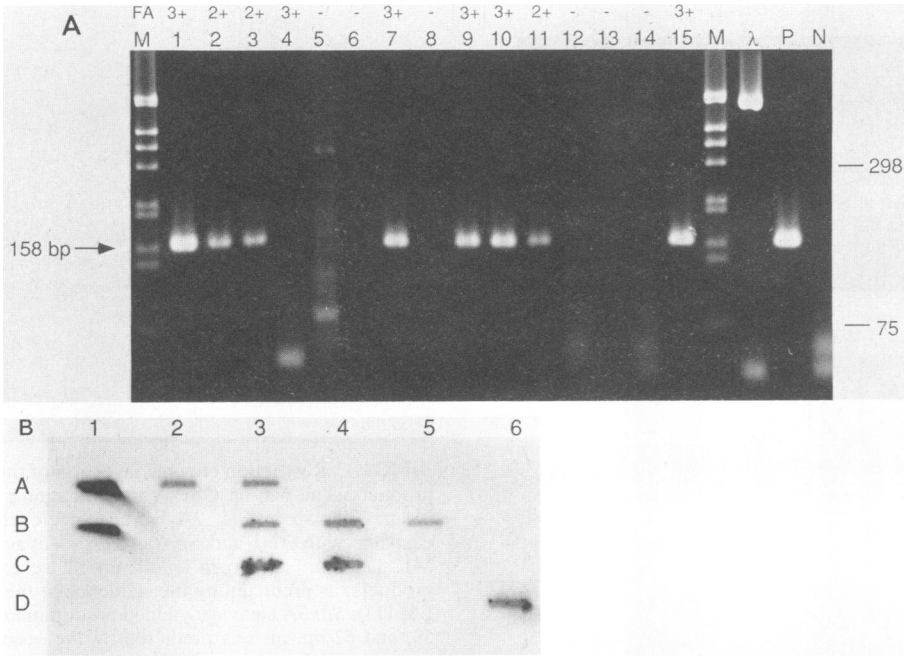

FIG. 5. PCR detection of *B. burgdorferi* in field-collected ticks. (A) Midguts were dissected from 15 female adult ticks from Nantucket, Mass., and subjected to analysis by both DFA staining and PCR amplification, using primers OSP-A2 and OSP-A4 as described in the text. The negative control contained all of the reaction components plus deionized water; the positive control contained DNA from strain B31. A control consisting of the reaction mixture plus bacteriophage lambda DNA and phage-specific primers was also included. The reaction products were electrophoresed in a 3% Nusieve–1% SeaKem agarose gel and detected by UV transillumination after being stained with ethidium bromide. DFA results were scored as 1+, 2+, 3+, or 4+, as given in the text. The results of the DFA staining for each sample are given above each lane. Lanes denoted N, P, and λ contain the negative, positive, and lambda controls, respectively. M denotes lanes containing the DNA size markers; representative marker sizes are shown at the right. (B) PCR products from the above specimens were slot blotted onto nylon membranes and detected with ^{32}P-end-labeled OSP-A3 as described in the text. The filter was washed and exposed to film between intensifying screens for 1 h at room temperature. Slots A1 to A6 correspond to lanes 1 to 6 in panel A, slots B1 to B6 correspond to lanes 7 to 12, and slots C1 to C3 correspond to lanes 13 to 15. Slots C4 and D6 contain positive controls. Slots C5 and C6 contain the negative and lambda controls, respectively. An 18-h exposure of the same blot revealed that slot A5 (lane 5, panel A) was positive (data not shown).

were positive by DFA; 7 of 14 were positive by PCR. The similar rates of infection obtained for divided and whole ticks suggested that sampling error did not significantly influence the number of infected ticks identified.

TABLE 1. Guts from field-collected nymphal *I. dammini* ticks divided equally and analyzed in double-blind fashion by DFA and PCR

Sample no.	Detection	
	DFA	PCR
1	−	−
2	−	−
3	−	−
4	3+	+
5	2+	+
6	−	−
7	−	−
8	−	−
9	−	−
10	3+	+
11	3+	+
12	4+	+
13	3+	+
14	−	−
15	1+	+

PCR analysis of desiccated dead specimens. We asked whether dead specimens represented material suitable for DNA amplification in our assay. Colony-derived nymphal ticks were examined by the DFA procedure; in this collection, 10 of 15 ticks were positive. Fourteen ticks from the same collection were allowed to die in a low-humidity environment 3 weeks prior to analysis. The dead whole ticks were processed as above (the brittleness of the dead specimens actually aided in their processing), amplification was carried out with primers OSP-A1 and OSP-A2, and the amplification products were detected with a ^{32}P-labeled OSP-3 probe. Nine of the 14 dead specimens gave positive results by PCR (data not shown). None of these specimens was considered suitable for analysis by immunofluorescence.

DISCUSSION

We have developed an assay based on the PCR for the detection of *B. burgdorferi* and have applied this technique to the detection of OSP-A-specific sequences in the arthropod vector *I. dammini*. Potential advantages of using PCR in this context include (i) the production of an amplified sequence that can be used to further characterize or classify the pathogen, (ii) the ability to detect the organism in dead specimens considered unsuitable for analysis by conventional means, and (iii) the ability to amplify multiple target

sequences within the same specimen. The last feature might be exploited in the analysis of *I. dammini* to detect other pathogens, such as *Babesia microti*, which shares this arthropod vector with *B. burgdorferi* in many enzootic areas (3, 25).

The use of the OSP-A gene of reference strain B31 as a target for amplification restricts the use of the current assay to the detection of those strains harboring a related sequence. While a 31-kilodalton OSP-A gene product is present in the great majority of the Northern American isolates examined to date (9–11), several investigators have recently described organisms isolated in the United States with a larger OSP-A protein that resemble those more commonly found in Europe (5, 15). One *I. dammini* isolate from Millbrook, N.Y. (Fig. 2, lane 9), had an OSP-A protein of 32.5 kilodaltons, as did an unusual *I. pacificus* isolate from northern California and one obtained from a German tick. None of these isolates produced a readily identified amplification product in our assay. The basis for this lack of reactivity is unknown; while the OSP-A proteins of both of the "atypical" North American isolates share antigenic determinants with that of B31 (5, 15), there may be insufficient nucleotide sequence homology in the primer binding sites to allow efficient amplification. Alternatively, primer binding sites may be lost due to delction mutation or placed into a remote location by recombination during antigenic variation (7, 27). Sequence analysis of the OSP-A genes of these variant strains is necessary to determine the basis for our results.

Our inability to detect sequence variation in the OSP-A-specific amplification products by restriction fragment length polymorphism analysis may be due to the small number of total nucleotides tested (40 of the total 646 nucleotides). However, considering that a number of these nucleotides are located in silent positions in the reading frame, several sequence variations might have been expected. This apparent conservation may result, in part, from the overall bias of the *B. burgdorferi* genome toward A-T richness, in which third-base codon substitutions of a G or C may not be tolerated at the level of codon choice (17). An interesting alternative explanation is the possibility that the spread of this organism in the North American continent may have been a relatively recent event. Though the results suggest that the OSP-A sequence in these isolates may be highly conserved, further analysis with other restriction enzymes and by DNA sequencing is necessary to determine the true extent of this conservation.

Recent experiments indicate that OSP-A-related sequences can be recovered from some, but not all, *B. hermsii* isolates (D. H. Persing, unpublished observations). This finding, together with the lack of a detectable amplification product in 3 of the 10 tested isolates, underscores the need to develop targets for amplification that are more highly conserved among isolates of *B. burgdorferi*. Examples of the latter might include genes encoding the flagellin proteins (19), certain sequences within the gene for the M_r-65,000 bacterial common antigen (33), structural genes for ribosomal subunit RNA (18), or more highly conserved sequences within the linear plasmid containing the OSP-A gene (29).

We have demonstrated that the amplification of *B. burgdorferi*-specific sequences using the PCR can be applied to the detection of the agent in field-collected ticks. While the application of this new technique represents an advance, especially in the analysis of dead specimens, the conventional methods of fluorescent-antibody staining and primary isolation in culture have served well in determining the prevalence of infection in enzootic areas. In contrast, the latter methods have been much less rewarding in the detection of the spirochete in infected human tissues (8, 12). It is hoped that the diagnostic advances that have been attained by using nucleic acid amplification techniques for the detection of other human pathogens will soon be realized for *B. burgdorferi* as well.

ACKNOWLEDGMENTS

We thank Paul Rys for superlative technical assistance, Debbie Beck and Gordon Terwilliger for help with protein gel electrophoresis and culture, and John Anderson, Steven Edberg, and Tom White for helpful discussions.

This study was supported in part by Public Health Service grants AI-26815 (to S.W.B.) and AI-19693 (to A.S.) from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Anderson, J. F., J. M. Doby, A. Coutarmanac'h, F. W. Hyde, and R. C. Johnson.** 1986. Differences antigeniques entre des souches de *Borrelia burgdorferi* isolees d'*Ixodes ricinus* en Bretagne. Med. Mal. Infect. **3**:171–175.
2. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde.** 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). J. Clin. Microbiol. **22**:36–38.
3. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, F. W. Hyde, and J. E. Myers.** 1987. Prevalence of *Borrelia burgdorferi* and *Babesia microti* in mice on islands inhabited by white-tailed deer. Appl. Environ. Microbiol. **53**:892–894.
4. **Anderson, J. F., and L. A. Magnarelli.** 1984. Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. Yale J. Biol. Med. **57**:627–641.
5. **Anderson, J. F., L. A. Magnarelli, and J. B. McAninch.** 1988. New *Borrelia burgdorferi* antigenic variant isolated from *Ixodes dammini* from upstate New York. J. Clin. Microbiol. **26**:2209–2212.
6. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
7. **Barbour, A. G., and C. F. Garon.** 1987. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. Science **237**:409–411.
8. **Barbour, A. G., and S. F. Hayes.** 1986. Biology of *Borrelia* species. Microbiol. Rev. **50**:381–400.
9. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152**:478–484.
10. **Barbour, A. G., and M. E. Schrumpf.** 1986. Polymorphisms of major surface proteins of *Borrelia burgdorferi*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **263**:83–91.
11. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **45**:94–100.
12. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308**:740–742.
13. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochete *Borrelia burgdorferi*. Mol. Microbiol. **3**:479–486.
14. **Bissett, M. L., and W. Hill.** 1987. Characterization of *Borrelia burgdorferi* strains isolated from *Ixodes pacificus* ticks in California. J. Clin. Microbiol. **25**:2296–2301.
15. **Bissett, M., W. Hill, W. Probert, and S. Kurashige.** 1988. An unusual isolate of *Borrelia burgdorferi* from a tick in California in Lyme Disease and Related Disorders. Ann. N.Y. Acad. Sci.

539:369–371.
16. **Burgdorfer, W.** 1984. Discovery of the Lyme spirochete and its relationship to the tick vector *Ixodes dammini*. 1984. Yale J. Biol. Med. **57**:515–520.
17. **deBoer, H. A., and R. A. Kastelein.** 1986. Biased codon usage: an exploration of its role in optimization of translation, p. 225–285. *In* W. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworth, Stoneham, Mass.
18. **DeLong, E. F., G. S. Wickham, and N. R. Pace.** 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science **243**:1360–1363.
19. **Gassmann, G. S., M. Kramer, U. B. Gobel, and R. Wallich.** 1989. Nucleotide sequence of a gene encoding the *Borrelia burgdorferi* flagellin. Nucleic Acids Res. **17**:3590.
20. **Lane, R. S., and W. Burgdorfer.** 1988. Spirochetes in mammals and ticks (Acari: Ixodidae) from a focus of Lyme borreliosis in California. J. Wildl. Dis. **24**:1–9.
21. **Latsavita, C. C., M. L. Wilson, V. P. Bernardi, A. Spielman, and R. D. Devlinger.** 1989. Rapid emergence of a focal epidemic of Lyme disease in coastal Massachusetts. N. Engl. J. Med. **320**:133–137.
22. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155**:335–350.
23. **Ou, C. Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **239**:295–297.
24. **Persing, D. H., and M. L. Landry.** 1989. In vitro amplification techniques for the detection of nucleic acids: new tools for the diagnostic laboratory. Yale J. Biol. Med. **62**:159–171.
25. **Piesman, J., T. N. Mather, G. J. Dammin, S. R. Telford III, C. C. Lastavica, and A. Spielman.** 1987. Seasonal variation of transmission risk of Lyme disease and human babesiosis. Am. J. Epidemiol. **126**:1187–1189.
26. **Piesman, J., T. N. Mather, R. J. Sninsky, and A. Spielman.** 1987. Duration of tick attachment and *Borrelia burgdorferi* transmission. J. Clin. Microbiol. **25**:557–558.
27. **Plasterk, R. H., M. I. Simon, and A. G. Barbour.** 1985. Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. Nature (London) **318**:257–263.
28. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
29. **Schwan, T. G., W. G. Simpson, M. E. Schrumpf, and R. H. Karstens.** 1989. Identification of *Borrelia burgdorferi* and *B. hermsii* using DNA hybridization probes. J. Clin. Microbiol. **27**:1734–1738.
30. **Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman.** 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annu. Rev. Entomol. **301**:439–460.
31. **Steere, A. C., T. F. Broderick, and S. E. Malawista.** 1978. Erythema chronicum migrans and Lyme arthritis: epidemiological evidence for a tick vector. Am. J. Epidemiol. **108**:312–332.
32. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–740.
33. **Thole, J. E., P. Hindersson, J. de Bruyn, F. Cremers, J. van der Zee, H. de Cock, J. Tommassen, W. van Eden, and J. D. van Embden.** 1988. Antigenic relatedness of a strongly immunogenic 65 kDA mycobacterial protein antigen with a similarly sized ubiquitous bacterial common antigen. Microb. Pathog. **4**:71–83.
34. **Wallis, R. C., S. E. Brown, and A. J. Main.** 1978. Erythema chronicum migrans and Lyme arthritis: field study of ticks. Am. J. Epidemiol. **108**:322–328.