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A POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF THE PARASITE *WUCHERERIA BANCROFTI* IN HUMAN BLOOD SAMPLES

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Abstract. To identify *Wuchereria bancrofti* DNA sequences that could be used as the basis for a simple and rapid parasite detection assay, a genomic library of *W. bancrofti* was constructed and screened for highly repeated DNA. The repeat found with the highest copy number was 195 basepairs (bps) long, 77% AT, and 300 copies per haploid genome. This sequence was designated the *Ssp* I repeat because it has a unique recognition site for that restriction endonuclease in all or most of the repeat copies. The *Ssp* I repeat DNA family is dispersed, genus-specific, and exists in all of the different geographic isolates of *W. bancrofti* tested. Based on DNA sequence analysis of this repeat, we have developed an assay to detect very small quantities of *W. bancrofti* DNA using the polymerase chain reaction (PCR). With this PCR assay, the *Ssp* I repeat was detected in as little as 1 pg of *W. bancrofti* genomic DNA (about 1% of the DNA in one microfilaria) added to 100 μl of human blood. The PCR assay also amplified *Ssp* I repeat DNA from geographic isolates of *W. bancrofti* from around the world but not from other species of filariae or from human or mosquito DNA. Microfilaria-positive human blood samples collected in Mauke, Cook Islands were shown to be *Ssp* I PCR-positive, while microfilaria-negative samples were PCR-negative. The specificity and sensitivity of the *Ssp* I PCR assay indicates that this approach has significant potential for improved screening of large human populations for active *W. bancrofti* infection.

The filarial parasite *Wuchereria bancrofti* is the major cause of lymphatic filariasis in tropical and subtropical regions of the world with an estimated 100 million people infected and an additional 900 million at risk of infection.[1]

Current methods of diagnosis of infection include identification of microfilariae by blood filtration, serodiagnosis by detection of an antibody to filarial antigen, and detection of circulating filarial antigen in the blood of infected patients.[1] Each method suffers from a number of limitations. Hemofiltration requires the collection of blood samples when microfilariae are present in the peripheral circulation (usually at night between 10:00 PM and 2:00 AM in regions where the infection is nocturnally periodic). This is not only problematic in obtaining the cooperation of the local population in community-based control programs, but also occult infections (infection in the absence of microfilariae) are not detected by this method. In addition, species identification is difficult in regions coendemic for *W. bancrofti* and *Brugia malayi*. Serodiagnosis, while sensitive, is relatively nonspecific and may be positive in subjects with exposure to filarial antigen without infection, in subjects with previous filarial infection, and in subjects infected with other helminths.[2] Assays that detect circulating *W. bancrofti* antigen enable the diagnosis of nocturnally periodic infections in day blood and the diagnosis of occult infections.[2,3] However, these assays fail to detect infection in some microfilaremic subjects.[4]

To investigate the possible efficacy of a DNA-based diagnostic test for *W. bancrofti* infection, a polymerase chain reaction (PCR) assay based on highly repeated DNA was developed. Previous studies have demonstrated the effectiveness of DNA probes derived from the *B. malayi Hha* I repeat,[5–9] and a PCR assay based on this repeat[10] for detection of single microfilaria in blood samples from infected individuals. Unlike *B. malayi*, which has > 30,000 copies of the tandemly arranged *Hha* I repeat per haploid genome, *W. bancrofti* has many dispersed repeat DNA families, each consisting of dozens to hundreds of copies. This paper describes the cloning, characterization, and diagnostic utility of the highest copy number repeat family found in *W. bancrofti*. This 195-basepair (bp) genus-specific repeat is termed the *Ssp* I repeat and exists in all geographic isolates of the genus *Wuchereria*. This sequence provides the basis of a PCR-based strategy for the detection of *W. bancrofti* DNA in the blood of infected patients.

MATERIALS AND METHODS

Isolation of DNA from parasites and construction of a genomic library. *Wuchereria bancrofti* var. pacifica microfilariae were collected from the blood of an infected Polynesian donor. All restriction endonucleases, ligases, and linkers used in these experiments were obtained from New England Biolabs (Beverly, MA) and used as described by the supplier. Cloning kits (λgt10) kits and Gigapack II Gold packaging kits were obtained from Stratagene (La Jolla, CA) and used as described by the manufacturer. Genomic *W. bancrofti* DNA was isolated from 50,000 microfilariae by digestion for 1 hr with 50 μg of proteinase K (Boehringer Mannheim, Indianapolis, IN) in a total volume of 200 μl. The digestion was extracted with phenol four times and chloroform/isoamyl alcohol (24:1) twice. The extracted DNA was then drop-dialyzed at room temperature for 3 hr.[11] The purified *W. bancrofti* genomic DNA was partially digested with the restriction endonucleases *Rsa* I and *Alu* I. Centricon-100 (Amicon, Beverly, MA) centrifugation was used to eliminate DNA fragments less than 100 bps. The purified DNA was ligated to 5′-phosphorylated *Eco* RI linkers with T4 DNA ligase, and then ligated to *Eco*RI-cleaved λgt10 vector DNA. For library construction, *Escherichia coli* C600

cells were infected with the λgt10 recombinant bacteriophages.

Isolation of a repeat DNA family from the *W. bancrofti* genomic library. *Wuchereria bancrofti* genomic DNA was labeled by random priming (Random Primed DNA Labeling Kit; United States Biochemicals, Cleveland, OH) using [α-^{35}S]dATP (Amersham, Arlington Heights, IL). The labeled DNA probe was purified using a Nuctrap Push Column (Stratagene) and used to screen the *W. bancrofti* genomic library. The λgt10 plaque lift filters were hybridized with [α-^{35}S]-labeled probe at 60°C overnight. Following hybridization, the filters were washed twice for 15 min at room temperature with 2× SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulfate (SDS) and once for 15 min at 60°C with 0.2× SSC/0.1% SDS. The positive plaques giving the strongest hybridization signals were selected for subcloning.

Subcloning the repeat DNA into the pCRII vector. Cloning kits (TA 2000) containing the plasmid pCRII were obtained from Invitrogen (San Diego, CA). All of the PCR reagents used in these experiments were obtained from Perkin-Elmer (Norwalk, CT). Positive plaques identified in the screening with total genomic DNA were amplified by PCR using a pair of primers flanking the cloning site of the λgt10 vector (#1231 and #1232; New England Biolabs). The PCR assays were carried out using the following conditions: denaturation for 10 min at 95°C; followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1.5 min at 72°C; followed by extension for 10 min at 72°C. The amplified PCR products were subcloned into the pCRII plasmid vector according to the manufacturer's protocol. The positive recombinant plasmid clones were identified using PCR. In this method, cells from white (recombinant) colonies were picked and amplified using the original λgt10 PCR primers. If the PCR product had the same size as the original lambda clone, it was chosen for DNA sequence analysis.

DNA sequence analysis of the repeat clones. Plasmid DNA was isolated from 17 pCRII/*W. bancrofti* repeat clones using the Plasmid Midi Kit from Qiagen (Chatsworth, CA). All of the repeat clones were sequenced by thermal cycle sequencing using the Vent$_R$(exo−) Sequencing Kit from New England Biolabs. The sequencing reactions were conducted using a thermal profile of 20 sec at 95°C, 20 sec at 50°C, and 20 sec at 72°C for 20 cycles. The sequence data were analyzed using the MacVector software package from Scientific Imaging Systems (New Haven, CT) and the sequence analysis package from Genetics Computer Group (Madison, WI). The most common repeat sequence selected in this screening was 195 bp in length and contained a single *Ssp* I restriction site.

Southern and dot-blot hybridizations. *Wuchereria bancrofti* genomic DNA and plasmid DNA were labeled nonradioactively using fluorescein-11-dUTP by random priming (Enhanced Chemiluminescence [ECL] Random Priming Labeling System; Amersham). The 17-mer internal oligonucleotide (Figure 1) was 3′-end labeled also using fluorescein-11-dUTP (ECL 3′-Oligolabeling System; Amersham). Southern hybridizations were performed using protocols provided in the ECL kits except that nitrocellulose membranes (Schleicher & Schuell, Keene, NH) were used instead of nylon membranes. The membranes were incubated in ECL prehybridization buffer for 2 hr at 50°C when random prime-labeled genomic or plasmid DNA was the probe, and at 37°C when the 3′ end-labeled 17-mer oligonucleotide was the probe. The filters were hybridized overnight with fluorescein-labeled probes at 50°C or 37°C, again depending on which probe was used. The protocols used for membrane washes, blocking, antibody incubations, and signal detection were as described by the manufacturer (Amersham).

For dot-blot hybridization experiments, DNA was filtered onto nitrocellulose membranes (Schleicher & Schuell). The filters were denatured using 0.5 M NaOH for 2 min, and then neutralized using 1.0 M Tris-HCl (pH 7.5) for 5 min, and 0.5 M Tris-HCl (pH 7.5)/1.5 M NaCl for 5 min. The filters were prehybridized, hybridized, washed, and detected using the same probes and protocols given above for Southern blots.

Developing the *Ssp* I PCR system. Based on the sequence of the *Ssp* I repeat, a pair of primers (NV-1 and NV-2) were designed for PCR amplification of the *Ssp* I repeat. The sequences are as follows: NV-1: 5′ CGTGATGGCATCAAAGTAGCG 3′ (21-mer); NV-2: 5′ CCCTCACTTACCATAAGACAAC 3′ (22-mer). These primers hybridize close to the ends of the 195-bp repeat and yield a 188-bp PCR amplification product. The PCR conditions using these two primers were optimized by titration of the concentrations of $MgCl_2$, deoxynucleotide triphosphates, primers, and *Taq* I polymerase. These titrations were conducted near the limit of detection for this PCR system (about 1 pg of *W. bancrofti* genomic DNA). The PCRs were conducted using

```
        10         20         30         40         50
        .          .          .          .          .
AGCGTGATGGCATCAAAGTAGCGTAAGGGAATTGTTTTTTTAATATTTTC
NV-1                                          Ssp I site

        60         70         80         90         100
        .          .          .          .          .
AAGTATGAATGGAATTTTTAGCAATTTTTTTGTTTATATTTTTATTTGAA

        110        120        130        140        150
        .          .          .          .          .
TTATTTTTTTTTTTGTTTGCTTGGTATAACCTTATTTTTTAATCTTTTTT
              Hybridization Probe

        160        170        180        190
        .          .          .          .
AATTTTTTTAGTTTTTTTGTTGTCTTATGGTAAGTGAGGGAATTC
                                        NV-2
```

45/195 = 23% G+C, 77% A+T
A-T Rich Middle Region: 19/139 = 14% G+C, 86% A+T
5′+ 3′ Ends: 25/54 = 46% G+C
PCR Primers: NV-1 (21-mer, 11/21 GC)
NV-2 (22-mer, 10/22 GC)
Hybridization Probe: 7/17 GC

FIGURE 1. Sequence of the *Ssp* I repeat DNA. The repeat is 195 basepairs in length and is 77% AT. The middle region of this repeat is especially AT-rich (containing only 14% GC). The underlined sequences at the ends of the repeat are the primers NV-1 1 and NV-2 used for *Ssp* I polymerase chain reaction (PCR) amplification. An internal oligonucleotide (a 17-mer, underlined) was chosen as a hybridization probe and was used in Southern blot hybridizations to ensure that the PCR products are the specific *Ssp* I repeat of *Wuchereria bancrofti.*

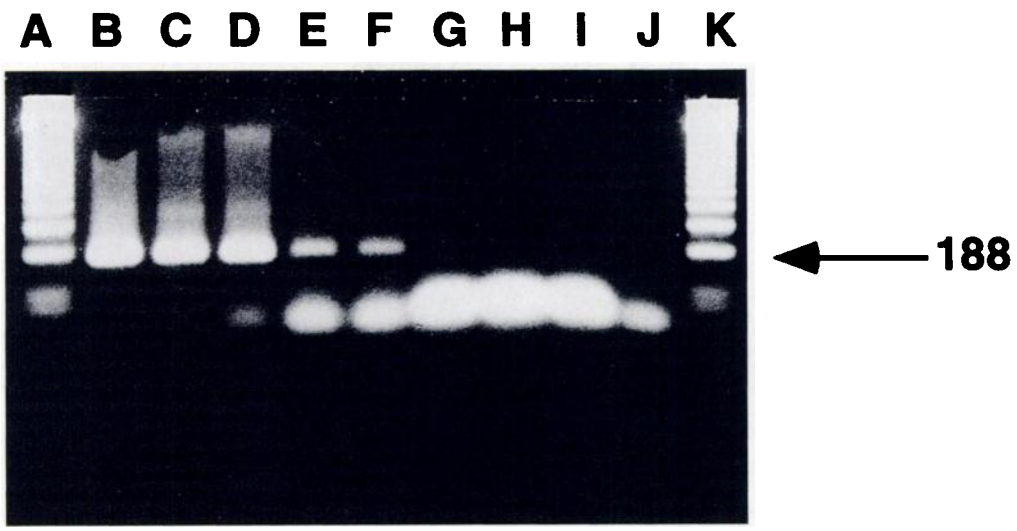

FIGURE 2. Sensitivity of the *Ssp* I polymerase chain reaction (PCR) system demonstrated by staining with ethidium bromide. Different amounts of *Wuchereria bancrofti* genomic DNA (1 ng to 0.01 pg) were used as the PCR template to test the sensitivity of the *Ssp* I PCR system using the optimal conditions (lanes B–J). Lanes A and K, 100-basepair (bp) ladder (molecular weight marker). Lanes B–I, *Ssp* I PCR amplification of 1 ng, 100 pg, 10 pg, 1 pg, 0.5 pg, 0.1 pg, 0.05 pg, and 0.01 pg of *W. bancrofti* genomic DNA. Lane J, no DNA template (negative PCR control). A positive PCR signal (a band at 188 bp) was observed even when only 0.05 pg of *W. bancrofti* genomic DNA was used as a template for PCR. The lowest band of DNA seen in lanes B–J is primer/dimer.

40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by a 72°C extension for 10 min. Following PCR, 20% of each sample was run on a 2.5% agarose gel and stained with ethidium bromide. To verify the identity of the 188-bp PCR amplification product, Southern blots of these gels were routinely performed and hybridized with the 17-mer internal oligonucleotide probe (Figure 1).

Detection of microfilariae in blood by the *Ssp* I PCR assay. Different numbers of *W. bancrofti* microfilariae from French Polynesia or Egypt were added to 50 μl of human blood. The samples were processed using the following protocol: 1) digestion with 15 μl of proteinase K (20 mg/ml) at 65°C for 3 hr; 2) extraction once with phenol, once with phenol/chloroform, and once with chloroform; and 3) dialysis against 0.1× TE (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) at room temperature for 4 hr. Each PCR included 5.0 μl of the dialyzed samples as templates. Following PCR, 20% of each sample was run on a 2.5% agarose gel and stained with ethidium bromide.

Detection of parasite DNA in blood and serum by the *Ssp* I PCR assay. Different amounts of *W. bancrofti* genomic DNA were added to 50 μl of human blood or serum. The samples were processed using the method described above and 5 μl of the processed samples were used as templates for PCR. Following PCR, 20% of each sample was run on a 2.5% agarose gel and stained with ethidium bromide.

Screening blood samples using the *Ssp* I PCR assay. Human blood samples of 100 μl were collected in vacutainers containing EDTA. Additional EDTA was added to a final concentration of 0.1 M and samples were stored at −20°C. Later, the blood samples were thawed and processed using the following protocol: 1) digestion with 15 μl proteinase K (20 mg/ml) at 65°C for 3 hr; 2) extraction once each with phenol, phenol/chloroform, and chloroform; and 3) dialysis against 0.1× TE at room temperature for 4 hr. Five microliters of the dialyzed samples were used as templates for the *Ssp* I PCR assay. Following PCR, 20% of each sample was run on a 2.5% agarose gel and stained with ethidium bromide.

Detection of microfilaremia in clinical specimens using the *Ssp* I PCR assay. In July 1992, blood samples were collected from 47 residents of the South Pacific island of Mauke, Cook Islands and were evaluated for infection with *W. bancrofti*. Blood samples were collected in EDTA vacutainers between 8:00 AM and noon when microfilarial density is at its peak in that area (unpublished data). Microfilarial density was assessed by standard 1-ml blood filtration through 3-μm pore size Nuclepore filters (Nucleopore Corp., Pleasanton, CA).[1] An additional 0.1 ml of blood was collected for the PCR assay and preserved in EDTA and frozen as described above. These samples were processed for PCR using the method described above for detecting microfilariae in blood. The samples were then tested with the *Ssp* PCR assay using the 40-cycle program described above. An additional 23 samples were collected in Indonesia from individuals shown to have circulating *B. malayi* microfilariae. These samples were collected, processed, and PCR-tested in exactly the same manner as the Cook Island samples.

Nucleotide sequence accession number. The DNA sequence data reported in this paper were submitted to GenBank® and received the accession number L20344.

RESULTS

Characterization of the *Ssp* I repeat DNA. Seventeen repeat clones were selected from screening the *W. bancrofti* λgt10 genomic library using total *W. bancrofti* genomic DNA as the probe. All of these clones were PCR-amplified and subcloned into the pCRII plasmid vector and sequenced. Two of the clones (WbT12 and WbT14) showed 98% sequence identity for a 195-bp region. The other 15 clones

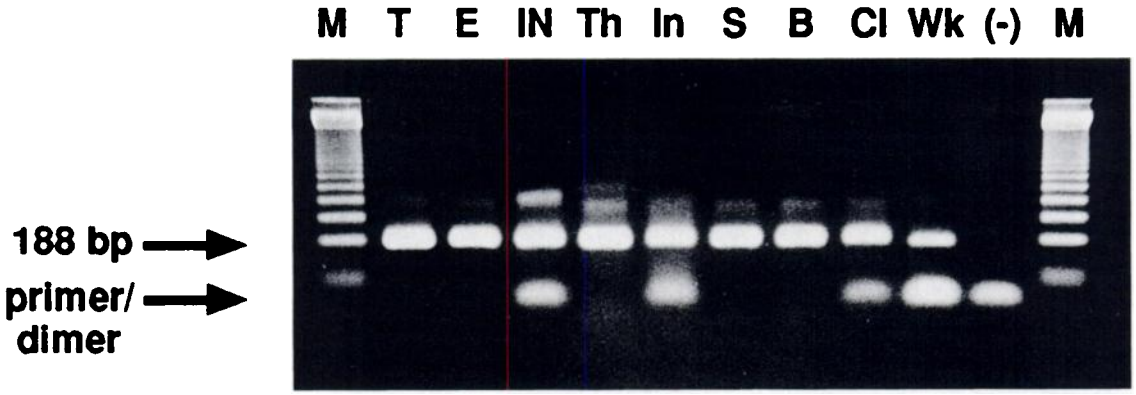

FIGURE 3. Polymerase chain reaction (PCR) amplification of the *Ssp* I repeat DNA from different geographic isolates of *Wuchereria bancrofti*. The DNA or microfilariae from different geographic isolates of *W. bancrofti* were amplified by the *Ssp* I PCR system. A 188-basepair (bp) band was amplified in all of these geographic isolates. The *Ssp* I repeat was also amplified from *W. kalimantani*. Lane M, 100-bp ladder (molecular weight marker); T, Tahiti; E, Egypt; IN, India; Th, Thailand; In, Indonesia; S, Sri Lanka; B, Brazil; Cl, Cook Islands; Wk, *W. kalimantani;* (−), negative control (no template).

showed no sequence similarity to WbT12 and WbT14. The regions flanking the 195-bp repeat fragment in WbT12 showed no sequence similarity to the regions flanking the repeat in WbT14, indicating that this repeat family is dispersed. The DNA sequence analysis of WbT12 and WbT14 showed a single *Ssp* I restriction site in both copies of this repeat. Thus, the sequences were designated the *Ssp* I repeat family. Base composition analysis indicates that this *Ssp* I repeat is very AT-rich (77% AT) (Figure 1). When equal amounts of DNA from all 17 repeat clones were spotted on a nitrocellulose filter and hybridized with *W. bancrofti* total genomic DNA, WbT12 and WbT14 gave the strongest signals. Six of the seventeen clones that gave the best sensitivity were labeled as probes to hybridize to serially diluted *W. bancrofti* genomic DNA on six dot-blots. The two *Ssp* I repeat DNA clones (WbT12 and WbT14) gave the best sensitivity and species-specificity. These clones could detect as little as 40 pg of *W. bancrofti* genomic DNA and did not hybridize to 5 ng of *Aedes polynesiensis* (mosquito), human, or *B. malayi* DNA. By comparison, single copy gene clones from *W. bancrofti* could detect (under identical hybridization conditions) as little as 12 ng of *W. bancrofti* genomic DNA. Thus, the copy number of the *Ssp* I repeat was estimated at 300 copies per haploid genome.

Optimizing the *Ssp* I PCR assay. A pair of PCR primers matching the two ends of the repeat was designed from the *Ssp* I sequence (Figure 1). The *Ssp* I PCR was found to give the maximum yield of product when 300 μM dNTP, 0.2 μM NV-1 primer, 0.2 μM NV-2 primer, 1.5 mM $MgCl_2$, and 0.04 U/μl of *Taq* DNA polymerase were used in the reaction.

Testing the sensitivity of the *Ssp* I PCR system. When *Ssp* I PCR assays were carried out using the optimal conditions, as little as 100 fg of *W. bancrofti* genomic DNA could be detected by staining with ethidium bromide (Figure

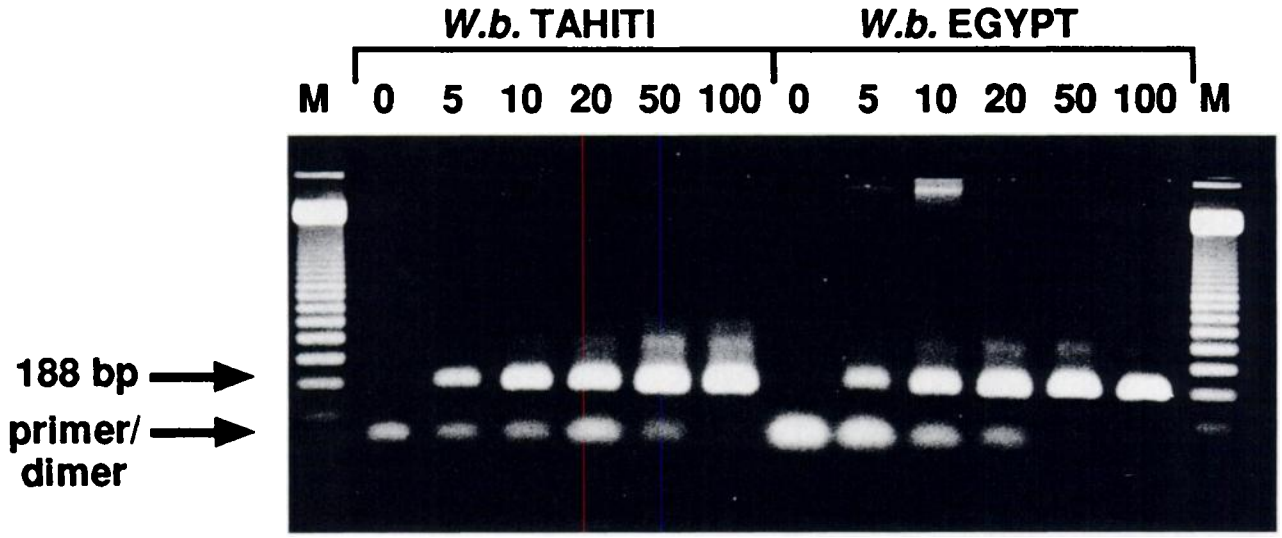

FIGURE 4. Detection of *Wuchereria bancrofti* (*W.b.*) microfilariae in blood using the *Ssp* I polymerase chain reaction (PCR) system. Different numbers (0–100) of French Polynesian or Egyptian *W. bancrofti* microfilariae were added to 50 μl of human blood. The samples were processed and the *Ssp* I PCRs were conducted as described. All of the samples containing microfilariae were PCR-positive. The blood samples without microfilariae were PCR-negative. The intensity of the ethidium bromide–stained bands increased as the number of microfilariae increased. Lanes M, 100-basepair (bp) ladder (molecular weight marker).

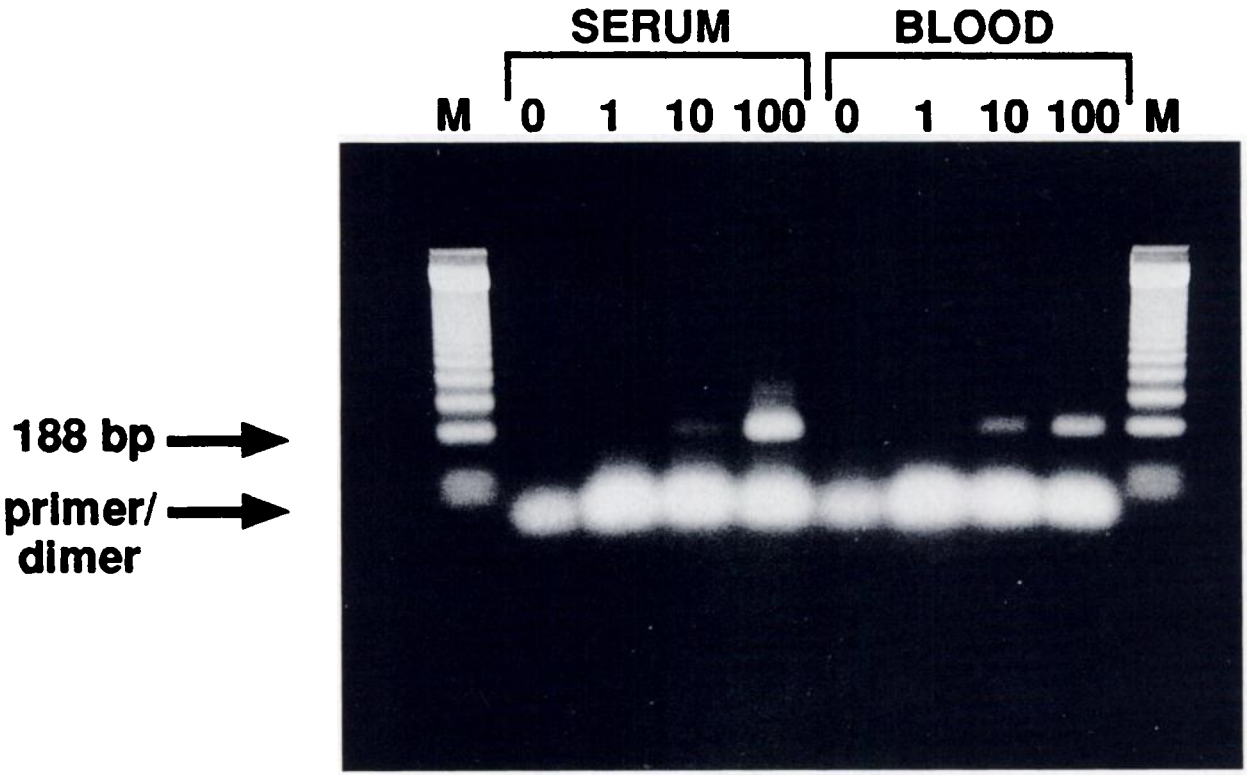

FIGURE 5. Detection of genomic *Wuchereria bancrofti* DNA added to blood or serum using the *Ssp* I polymerase chain reaction (PCR) system. Different amounts (0–100 pg) of *W. bancrofti* genomic DNA were added to 50 μl of human blood or plasma. The samples were processed and the *Ssp* I PCRs were conducted as described. All of the blood and plasma samples containing *W. bancrofti* DNA were PCR-positive; the blood and plasma samples without *W. bancrofti* DNA were PCR-negative. Lanes M, 100-basepair (bp) ladder (molecular weight marker). The 188-bp *Ssp* I PCR product is not visible in the 1 pg lanes in this photograph. The bands were visible in the original photograph, however.

2), and 50 fg of *W. bancrofti* genomic DNA could be detected on a Southern blot when hybridized with a 17-mer internal oligonucleotide probe of the *Ssp* I repeat. The location and sequence of this internal oligonucleotide probe is shown in Figure 1. The Southern blot also proved that the amplified PCR products were in fact the *Ssp* I repeat.

Testing the specificity of the *Ssp* I PCR system. The *Ssp* I repeat family was successfully amplified from DNA or microfilariae from all of the geographic isolates of *W. bancrofti* that were tested (Egypt, India, Thailand, Indonesia, Sri Lanka, Brazil and the Cook Islands) (Figure 3). This repeat was also amplified from *W. kalimantani*, the only other species in the genus *Wuchereria* (Figure 3). *Wuchereria kalimantani* is found only in *Presbytis cristatus* monkeys on the island of Kalimantan (Borneo) in Indonesia. When the *Ssp* I PCR system was used to amplify 16–50 ng of DNA from other organisms, including humans, *Ae. polynesiensis, Dirofilaria immitis, B. malayi, B. pahangi, B. timori, Acanthocheilonema vitae, Loa loa, Onchocerca volvulus, Litomosoides carinii,* and *Caenorhabditis elegans,* no amplification was seen both by staining with ethidium bromide and by hybridization with the 17-mer internal oligonucleotide probe (Figure 1). These data indicate that the *Ssp* I repeat family is genus-specific and that the repeat is found in *W. bancrofti* isolates throughout the world.

Detection of microfilariae in blood using the *Ssp* I PCR system. One of the goals in developing the *Ssp* I PCR system was to reliably detect very low numbers of microfilariae in blood samples. A reconstruction experiment was conducted by adding different numbers of French Polynesian or Egyptian *W. bancrofti* microfilariae to 50 μl of human blood. The samples were processed using proteinase K digestion, organic extraction, and dialysis. Five microliters (1/10 volume) of each of the dialyzed samples were used as a template for *Ssp* I PCR. A very strong signal could be observed when only five microfilariae were added to 50 μl of blood (Figure 4). Since only 1/10 of the sample was used for PCR, these data indicate that the assay should detect about one microfilaria in 100 μl or more of blood. The increased intensity of the ethidium bromide–stained bands as the number of microfilariae increased suggests the possibility of developing a semi-quantitative PCR assay. When the PCR signals of the French Polynesian and Egyptian microfilariae added to blood are compared, the intensity of the signal is virtually the same. This indicates that the *Ssp* I repeat family exists in similar copy number in these two geographic isolates of *W. bancrofti.*

Detection of parasite DNA in blood and serum using the *Ssp* I PCR assay. Reconstruction experiments were carried out to investigate the use of the *Ssp* I PCR system to detect parasite DNA in blood and serum samples. Different amounts of genomic *W. bancrofti* DNA were added to 50 μl of human blood or serum. These samples were processed using the method described above. When 5 μl (1/10 volume)

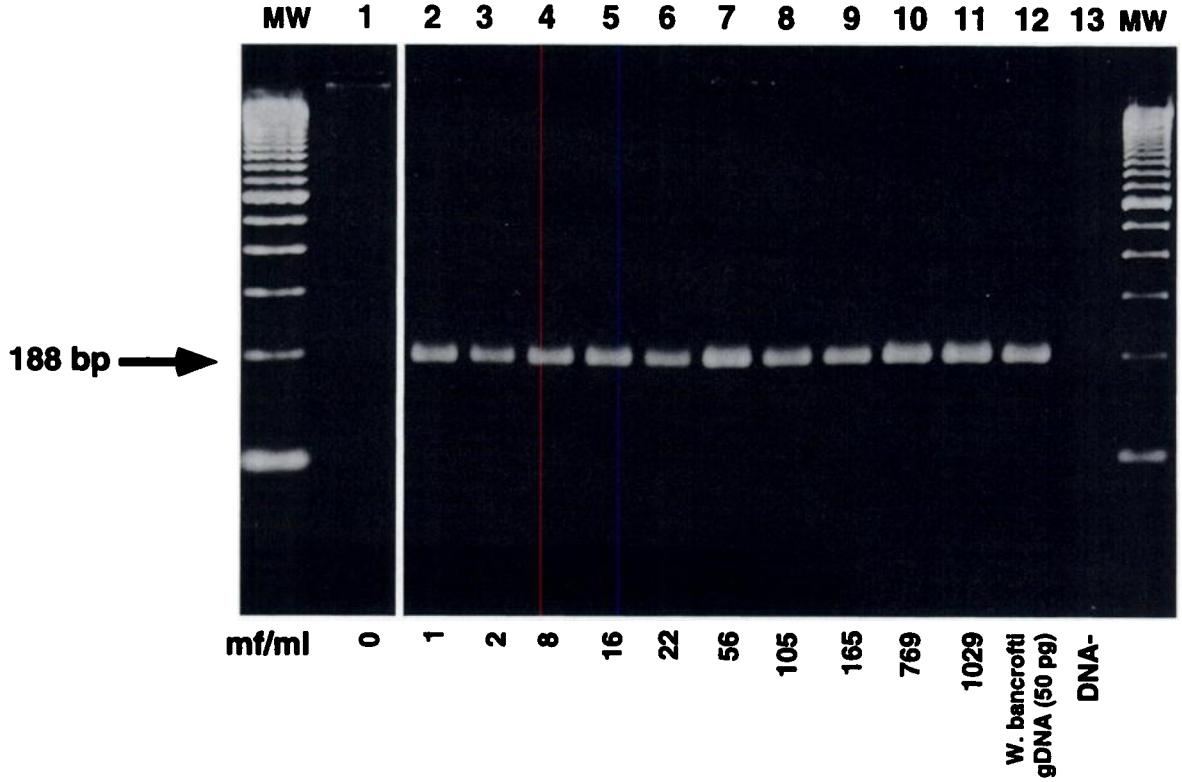

FIGURE 6. Detection of *Wuchereria bancrofti* infection in human blood samples using the *Ssp* I polymerase chain reaction (PCR) system. A total of 100 μl of blood from each of 47 residents of the *W. bancrofti*-endemic island of Mauke, Cook Islands, was subjected to analysis using the *Ssp* I PCR system. A 188-basepair (bp) band was visible in all 10 subjects with microfilaria (mf) counts ranging from 1 to 1,029/ml (lanes 2–11). The PCR of a blood sample from one of the amicrofilaremic subjects from the endemic area has no band visible (lane 1). The negative results from the other 36 residents without microfilariae (all negative for circulating antigen[4]) are not shown. Lane 12, PCR-positive control with 50 pg of *W. bancrofti* genomic DNA (gDNA); lane 13, PCR-negative control (no DNA added to 100 μl of blood); lanes MW, 100-bp ladder.

of the dialyzed samples were used as a template for PCR, a band was observed when as little as 1.0 pg of *W. bancrofti* DNA was added to the blood or serum (Figure 5).

Detection of microfilaremia in clinical specimens using the *Ssp* I PCR assay. Blood samples were collected from 47 residents of the *W. bancrofti*-endemic island of Mauke, Cook Islands in the South Pacific. Microfilaria counts ranged from 0 to 1,029/ml of blood. For each resident, 100 μl of blood was processed and subjected to analysis using the *Ssp* I PCR system as described in the Materials and Methods. A 188-bp band was visualized on ethidium bromide–stained agarose gels for all 10 subjects who had microfilarial counts of 1–1,029/ml (Figure 6). All 37 individuals without microfilaremia (and who were also circulating antigen–negative) were negative using the *Ssp* I PCR assay. These results were confirmed when the PCR products were subjected to Southern blot hybridization with the *Ssp* I 17-mer internal hybridization probe. It should be noted that the intensity of the PCR signal obtained from the individual with one microfilaria/ml of blood was comparable to the signal obtained from the individual with 1,029 microfilariae/ml of blood (Figure 6), suggesting that fewer PCR cycles will be necessary to develop a semi-quantitative format for the *Ssp* I PCR assay.[12]

To test the specificity of the assay further, blood samples were collected from 23 microfilaria-positive Indonesians infected with *B. malayi* (50–300 microfilariae/ml). All 23 of these samples were negative when tested with the *Ssp* I PCR assay although they were positive when tested by the *Hha* I *B. malayi* PCR assay.[10]

DISCUSSION

The sensitivity and specificity of the *Ssp* I PCR system has demonstrated its potential use as a diagnostic tool. The species-specificity of this assay could prove advantageous in a variety of circumstances. In some endemic areas, mosquito vectors carry more than one species of filarial parasite. The *Ssp* I system could be used in French Polynesia to specifically detect the mosquitoes infected with *W. bancrofti* and not *D. immitis* since these two species of filariae are carried by the same *Aedes* mosquito vector. This *Wuchereria*-specific PCR assay may also prove useful in specifically diagnosing bancroftian infection in humans in regions where other species of filarial parasites are coendemic with *W. bancrofti* (e.g., *B. malayi, L. loa, Mansonella perstans*). For example, in regions of Indonesia or Thailand where *W. bancrofti* and *B. malayi* are coendemic, the *Ssp* I PCR system could be used to detect bancroftian infections, while the *Hha* I PCR system[10] could be used to detect malayan infection.

The results presented in this paper demonstrate that the

Ssp I PCR assay can be used to detect very low numbers of microfilariae in blood samples. Samples with as few as one microfilaria/ml of human blood were detected in repeated trials, even though only 0.1 ml of blood was processed for each PCR assay. This result, coupled with our demonstration that as little as 1 pg of *W. bancrofti* DNA could be detected in 0.1 ml of blood, suggests the possibility of detecting *W. bancrofti* infection in situations where microfilaria cannot be found in the blood. Indeed, preliminary experiments indicate that the *Ssp* I PCR assay is positive in day blood samples in areas where infection is nocturnally periodic, although these samples are likely to have small numbers (less than one per 5–10 ml) of microfilariae even in the daytime. It can also be positive in some individuals who are amicrofilaremic but positive for circulating filarial antigen. While further research will be necessary to validate these observations, it is likely that this assay will prove to be a useful diagnostic tool that will complement the other available diagnostic methods.

While not yet validated in large-scale clinical trials, the potential applications of the *Ssp* I PCR assay in the field include: 1) quantitative assessment of parasite burdens in human blood and in infected mosquitoes; 2) the ability to detect DNA in the absence of circulating microfilariae; 3) the ability to detect DNA in day blood of patients with nocturnally periodic strains of *W. bancrofti;* and 4) the ability to specifically identify *W. bancrofti* parasites in human pathology samples when the speciation has been difficult based on morphologic evidence alone. Thus, the identification, characterization, and PCR-based utilization of the *Ssp* I repeat DNA provides the potential to improve the specific diagnosis of active *W. bancrofti* infection.

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