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Rapid Detection of *Serpulina hyodysenteriae* in Diagnostic Specimens by PCR†

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A PCR assay for the detection of *Serpulina hyodysenteriae* in diagnostic specimens was developed on the basis of sequence analysis of a recombinant clone designated pRED3C6. Clone pRED3C6, which contained a 2.3-kb DNA fragment unique to *S. hyodysenteriae*, was identified by screening a plasmid library of *S. hyodysenteriae* isolate B204 genomic DNA in *Escherichia coli* by colony immunoblot with the mouse monoclonal antibody 10G6/G10, which was produced against cell-free supernatant antigens from the same isolate. Southern blot analysis of *Hin*dIII-digested genomic DNA of *S. hyodysenteriae* serotypes 1 through 7 and of four weakly beta-hemolytic intestinal spirochetes, including *Serpulina innocens*, with the 2.3-kb DNA fragment of pRED3C6 indicated that the cloned sequence was present exclusively in the seven serotypes of *S. hyodysenteriae*. An oligonucleotide primer pair for PCR amplification of a 1.55-kb fragment and an internal oligonucleotide probe were designed and synthesized on the basis of sequence analysis of the 2.3-kb DNA fragment of pRED3C6. Purified genomic DNAs from reference isolates of *S. hyodysenteriae* serotypes 1 through 9, *S. innocens*, weakly beta-hemolytic intestinal spirochetes belonging to genotypic groups distinct from those of reference *Serpulina* spp., other cultivable reference isolates of the order *Spirochaetales*, and enteric bacteria including *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., and *Bacteroides vulgatus* were amplified with the oligonucleotide primer pair in a hot-start PCR. The 1.55-kb products were obtained only in the presence of genomic DNA from each of the nine serotypes of *S. hyodysenteriae*. The specificity of the 1.55-kb products for *S. hyodysenteriae* was confirmed on the basis of production of a restriction endonuclease pattern of the PCR products identical to the predicted restriction map analysis of pRED3C6 and positive hybridization signal with the *S. hyodysenteriae*-specific internal oligonucleotide probe. By using total DNA obtained from normal swine feces inoculated with decreasing concentrations of *S. hyodysenteriae* cells, the sensitivity of the PCR assay was calculated to be between 1 and 10 organisms per 0.1 g of feces. The PCR assay was 1,000 times more sensitive than conventional culture of dysenteric feces on selective medium. There was complete agreement between the results of PCR assays and anaerobic culture on selective agar medium with diagnostic specimens (n = 9) obtained from six farms on which there were cases with clinical signs suggestive of swine dysentery. Detection of *S. hyodysenteriae* by PCR amplification of DNA has great potential for rapid identification of *S. hyodysenteriae* in diagnostic specimens.

Swine dysentery is a highly contagious diarrheal disease of growing and finishing swine which causes an estimated loss of $2.4 million monthly to Iowa pork producers (28). The disease is caused by *Serpulina hyodysenteriae*, and nine different serotypes have been recognized worldwide (22). The diagnosis of swine dysentery is based on herd history, clinical signs, observation of characteristic intestinal lesions, and isolation of *S. hyodysenteriae* from feces or the intestine by using selective agar medium incubated anaerobically for 2 to 4 days (4). Laboratory confirmation of *S. hyodysenteriae* identification by culture is based upon colony morphology, pattern and intensity of hemolysis, and other growth characteristics, all of which are very similar for the nonpathogenic *Serpulina innocens*, a common inhabitant of the swine colon (16). As a result, a definitive diagnosis of swine dysentery can be very challenging, particularly when the disease occurs on farms where weakly beta-hemolytic intestinal spirochetes (WBHIS) are present in the swine population.

The development of sensitive and specific methods for rapid detection of *S. hyodysenteriae* in diagnostic specimens is needed. Amplification of specific DNA sequences by PCR provides a highly sensitive and specific tool for the detection of enteropathogenic bacteria directly from feces without the need for culture (27). In this report we describe the development of a specific and sensitive nucleic acid-based assay for rapid detection of *S. hyodysenteriae* in diagnostic specimens.

(A preliminary report of these findings was presented at the 36th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, Las Vegas, Nev., 1993, and the 53rd Annual Meeting of the American Society for Microbiology, North Central Branch, Ames, Iowa, 1993. This report represents a portion of a thesis submitted by R. O. Elder to the University of Nebraska—Lincoln in partial fulfillment of the requirements for the M.S. degree.)

MATERIALS AND METHODS

Bacterial strains. *S. hyodysenteriae* B78 serotype 1 (ATCC 27164 [16, 17]), B204 serotype 2, B169 serotype 3, and A1 serotype 4 were obtained from J. M. Kinyon, College of Veterinary Medicine, Iowa State University, Ames. Reference *S. hyodysenteriae* strains B234 serotype 1, B8044 serotype 5, B6933 serotype 6, and AcK 300/8 serotype 7 were provided by L. A. Joens, Department of Veterinary Science, University of Arizona, Tucson. Reference strains FM-88-90 serotype 8 and FMV 89-3323 serotype 9 were provided by M. Jacques, Faculté

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de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada (22). The reference isolates for WBHIS, *S. innocens* isolates B256 (ATCC 29796 [16]) and 4/71, were obtained from the American Type Culture Collection, Rockville, Md., and T. B. Stanton, National Animal Disease Center, Ames, Iowa, respectively. A total of 13 field isolates representing three genotypic groups of WBHIS distinct from *S. innocens* were obtained from porcine feces, porcine rectal swabs, and porcine colonic mucosal scrapings submitted either to the Veterinary Diagnostic Center, University of Nebraska—Lincoln, or to Agriculture Canada, Saint-Hyacinthe, Québec, Canada (20, 30). WBHIS isolates B359 and B1555a were obtained from J. M. Kinyon, and isolate D9201243A was provided by R. L. Walker, California Veterinary Diagnostic Laboratory System, University of California, Davis. WBHIS isolate 16 (ATCC 49776 [15]), obtained from a human immunodeficiency virus-positive homosexual male with diarrhea, was provided by R. M. Smibert, Virginia Polytechnic Institute, Blacksburg. *Treponema succinifaciens* isolate 6091 (ATCC 33096 [6]) and *Bacteroides vulgatus* (ATCC 31376) were obtained from the American Type Culture Collection. Chromosomal DNA from *Spirochaeta aurantia* was provided by E. P. Greenberg, University of Iowa. *Treponema pallidum* chromosomal DNA was provided by M. V. Norgard, University of Texas Health Science Center, Houston. Chromosomal DNA from representative strains of each of the 10 genetic groups in the family *Leptospiraceae* (including *Leptospira biflexa* serovars patoc, semaranga, and codice; *Leptospira interrogans* serovars icterohaemorrhagiae, fortbragg, ballum, celledoni, lyme, and borincana; and *Leptonema illini* serovar illini) were provided by R. L. Zuerner, National Animal Disease Center. Chromosomal DNA from *Borrelia burgdorferi*, *Campylobacter coli*, and *Campylobacter hyointestinalis* were provided by M. P. Murtaugh, University of Minnesota, St. Paul. Isolates of *Salmonella choleraesuis* and *Salmonella typhimurium* were provided by P. J. Fedorka-Cray, National Animal Disease Center. *Escherichia coli* DH5α was purchased from a commercial source (GIBCO-BRL, Gaithersburg, Md.).

Medium and growth conditions. For isolation of genomic DNA, cultures of *Serpulina* spp., WBHIS, and *T. succinifaciens* were propagated in prereduced anaerobically sterilized Trypticase soy broth supplemented with 0.5% (wt/vol) glucose (Sigma Chemical Co., St. Louis, Mo.), 0.05% (wt/vol) cysteine hydrochloride monohydrate (Sigma), 1.0% (wt/vol) yeast extract (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Md.), 2.0% (vol/vol) bovine fetal serum (HyClone Laboratories, Inc., Logan, Utah), 0.2% (wt/vol) sodium bicarbonate, and 5.0% (vol/vol) sterile porcine fecal extract as previously described (18), except that 1% (vol/vol) of room air was injected at the time of inoculation (35). Broth cultures were grown to late logarithmic phase in 5-ml volumes in Hungate tubes or in 250-ml volumes in serum bottles. Cultures were stirred constantly with a magnetic stirrer at 37°C under a 10% hydrogen–10% carbon dioxide–80% nitrogen atmosphere for 48 to 72 h. Cultures of *Salmonella* spp., *B. vulgatus*, and *E. coli* were grown at 37°C with shaking in Luria-Bertani broth and harvested in late logarithmic phase.

Chromosomal DNA isolation. Chromosomal DNA was purified as previously described (30) except that the final pellet was resuspended in sterile H_2O to a final concentration of 12.5 ng/μl.

Monoclonal antibody production. BALB/c mice, 8 to 10 weeks old, were immunized intraperitoneally with 100 μg of cell-free supernatant antigens from *S. hyodysenteriae* B204, concentrated 10 times with a YM5 Diaflow ultrafilter (Amicon, Beverly, Mass.), and mixed with equal volumes of Freund's complete adjuvant (8). Identical booster injections containing 50 μg of cell-free supernatant antigens in Freund's incomplete adjuvant were given 14, 28, and 42 days later. Four days after the last booster injection, spleen cells were harvested and fused with SP 2/0 cells by using 50% polyethylene glycol. Hybridomas producing antibodies that reacted with cell-free supernatant antigens from *S. hyodysenteriae* B204 by enzyme-linked immunosorbent assay were cloned by limiting dilution and stabilized before injection into mice for ascites production (11).

Library construction and recombinant screening. A recombinant plasmid library of *S. hyodysenteriae* B204 in *E. coli* DH5α was constructed as previously described (17a). Purified chromosomal DNA was partially digested with *Hin*dIII (Stratagene, La Jolla, Calif.) and ligated to plasmid vector pUC18 (GIBCO-BRL). Transformed *E. coli* DH5α cells were reacted with monoclonal antibodies as described previously (40). Replica membranes (catalog no. HATF 137 50; Millipore Corp., Bedford, Mass.) were lysed in chloroform vapor and then incubated overnight in lysis-blocking solution (5% nonfat dry milk, 0.5 M $MgCl_2$, 40 mg of lysozyme per ml, 100 mg of chloramphenicol per ml, 2 mg of DNase per ml) at room temperature. Membranes then were incubated sequentially at room temperature with ascites fluid for 2 h, biotin-labeled goat anti-mouse immunoglobulin A plus immunoglobulins G and M [heavy and light chain]) antibody (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md.) for 1 h, peroxidase-labeled streptavidin (Kirkegaard and Perry) for 45 min, and 4-chloro-1-naphthol (Kirkegaard and Perry) for 5 min. Five 5-min washes with wash buffer (1 M Tris base, 2 M NaCl, 5% nonfat dry milk, 0.05% Nonidet P-40 [pH 7.5]) were performed between each incubation step. One immunopositive clone, designated pRED3C6, was identified on the basis of development of a dark purple precipitate.

Plasmid and insert DNA isolation and sequencing. The recombinant plasmids were isolated (Magic Minipreps; Promega Corp., Madison, Wis.) and digested with the restriction enzyme *Hin*dIII (GIBCO-BRL), and the DNA fragments were separated by electrophoresis in a 0.8% agarose gel with TAE running buffer (16 mM Tris base, 8 mM sodium acetate, 1 mM EDTA [pH 7.5]) containing 0.66 μg of ethidium bromide per ml. The resulting bands were visualized and photographed under UV light with a Polaroid MP 4 land camera. Recombinant DNA bands were excised from the gels with a razor blade, isolated (Geneclean II; Bio-Rad, Richmond, Calif.), and subjected to the restriction enzymes *Acc*I, *Alu*I, *Eco*RI, *Dra*I, *Hae*III, *Ssp*I, and *Xba*I (GIBCO-BRL). The resulting fragments were purified and subcloned into vector pUC18 for sequencing with a model 4000 DNA sequencer (Li-Cor, Inc., Lincoln, Nebr.). Sequencing data were analyzed and assembled according to the program manual for the Genetics Computer Group package version 7 (April 1991).

Southern blotting. Chromosomal DNAs (approximately 2 μg each) from *S. hyodysenteriae* serotypes 1 to 7, *S. innocens* B256 and 4/71, WBHIS B359 and B1555a, and *T. succinifaciens* were digested with *Hin*dIII, electrophoretically separated on a 0.8% agarose gel, and transferred by capillary diffusion (34) to nylon membranes (Hybond-N; Amersham, Arlington Heights, Ill.). Prehybridization, hybridization, and washing steps with a recombinant DNA fragment obtained from the immunopositive clone pRED3C6 labeled with [α-^{32}P]dCTP by using an oligolabeling kit (Pharmacia LKB Biotechnology, Piscataway, N.J.) were performed as previously described (30). For slot blot analysis, purified genomic DNAs from cultivable reference isolates of the order *Spirochaetales* (including *S. hyodysenteriae* serotypes 1 through 9, *S. innocens* B256 and 4/71, 16 isolates of WBHIS belonging to three genotypic groups distinct

from *S. innocens*, *S. aurantia*, *Treponema* spp., *B. burgdorferi*, and representatives of each of the 10 genetic groups of the family *Leptospiraceae*) as well as enteric bacteria including *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *B. vulgatus* were applied to nylon membranes (Zeta-probe; Bio-Rad) by using a microfiltration apparatus (Bio-Dot SF; Bio-Rad). Prehybridization, hybridization, and washing steps with a recombinant DNA fragment obtained from the immunopositive clone pRED3C6 labeled with [α-^{32}P]dCTP were carried out as previously described (30).

Primers and internal probe for PCR and Southern blot analysis. An oligonucleotide primer pair (positive-sense 5′-GGTACAGGCGGAAACAGACCTT-3′ and negative-sense 5′-TCCTATTCTCTGACCTACTG-3′) and an internal *S. hyodysenteriae*-specific oligonucleotide probe (positive-sense 5′-TAGGGGCTGCTGTTCTAGCTGTAAATGC-3′) were designed and synthesized (Integrated DNA Technologies, Inc., Coralville, Iowa) on the basis of results of DNA sequence analysis of the recombinant DNA fragment of the immunopositive clone pRED3C6. The primers were used for amplification of either purified chromosomal DNAs or total DNA extracted either from normal porcine feces inoculated with *S. hyodysenteriae* cells or from porcine feces, porcine rectal swabs, and porcine colonic mucosal scrapings obtained from animals with clinical swine dysentery. The DNA was amplified by a hot-start PCR as described by the manufacturer (GeneAmp PCR System 480; Perkin-Elmer, Norwalk, Conn.) in a total volume of 75 μl containing 4 mM $MgCl_2$; 1× PCR buffer; 0.2 mM (each) dATP, dCTP, dGTP, and dTTP (Perkin-Elmer Cetus); 75 pmol of primers; and 1.5 U of *Taq* DNA polymerase (Perkin-Elmer Cetus) in sterile filtered autoclaved water. Initial denaturing was for 60 s at 95°C and was followed by 30 cycles (60 s at 65°C and 120 s at 72°C). The amplified products were visualized in 1.25% agarose gels run at 3 V/cm and stained with ethidium bromide. Southern blots were prehybridized and hybridized each for 1 h at T_m − 10°C (10°C below midpoint temperature) with an internal *S. hyodysenteriae*-specific oligonucleotide probe 5′ end labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase (Pharmacia) as previously described (26). Washes consisted of 1× SSPE (3.6 M NaCl, 0.2 M NaH_2PO_4, 0.02 M EDTA [pH 7.7]) with 0.1% sodium dodecyl sulfate (SDS) (three times for 5 min each at room temperature and once for 5 min at T_m − 10°C). The membranes were exposed to X-Omat AR Cronex radiograph film (Eastman Kodak Company, Rochester, N.Y.) in a cassette with Lightning Plus intensifying screens (DuPont, Wilmington, Del.) at −70°C.

PCR detection of *S. hyodysenteriae* in porcine feces. The sensitivity of PCR detection of *S. hyodysenteriae* in porcine feces was determined by two separate methods. In the first method, 10-fold serial dilutions of spirochete broth cultures were added to constant volumes of undiluted normal porcine feces in two separate experiments. The sensitivity of the PCR assay was estimated on the basis of the numbers of spirochete cells in the original culture as determined with a Petroff-Hauser cell counting chamber. Briefly, sterile tubes containing 0.1 g of normal feces were inoculated with 1 ml containing either sterile phosphate-buffered saline (PBS) (negative control) or serial 10-fold dilutions of *S. hyodysenteriae* B204 cells in sterile PBS, in concentrations ranging from 10^5 to 10^{-2}, and vortexed for 5 min. The samples were allowed to stand for 10 min, and then the supernatant fraction (approximately 0.9 ml) was drawn off and processed for total DNA extraction as described by the manufacturer (Nucleon DNA extraction kit; Scotlab, Shelton, Conn.) except that the samples were heated at 100°C for 15 min prior to the cell lysis step and 5 M sodium perchlorate (for deproteinization) was replaced by 100 μg of proteinase K per ml. Total DNA from each tube was used for PCR amplification followed by agarose gel electrophoresis and Southern blot analysis with the *S. hyodysenteriae*-specific oligonucleotide probe. In the second method, feces collected from two untreated animals at the onset of swine dysentery were serially diluted 10-fold (to a concentration of 10^{-1} to 10^{-12}) in 2-ml volumes of sterile PBS. One-milliliter fractions from each dilution were then processed for determination of the total numbers of viable *S. hyodysenteriae* organisms by a plate counting method and for detection of *S. hyodysenteriae*-specific products by the PCR assay, respectively. For the plate counting method, a total of 10 drops of 10 μl each were placed onto freshly made BJ selective medium, and the number of CFU per 0.1 ml was determined after incubation at 42°C in the GasPak Anaerobic System (BBL) for 9 days. One-milliliter fractions from each 10-fold dilution were processed for total DNA extraction and PCR detection of *S. hyodysenteriae*, as described above. The specificity of the PCR assay for detection of *S. hyodysenteriae* in diagnostic specimens was compared with that of conventional bacteriological culture on BJ medium incubated anaerobically at 42°C for 10 days. Porcine feces (n = 3), porcine rectal swabs (n = 2), and porcine colonic mucosal scrapings (n = 4) obtained from six different farms where clinical signs of swine dysentery were reported by the referring veterinarians (7) were processed for PCR assays and cultures. For PCR assays, total DNA was extracted from 100 μl of supernatants from either dysenteric porcine feces or porcine colonic mucosal scrapings, as described above. Rectal swabs were mixed with 1 ml of sterile PBS for 2 min, and the total DNA was extracted from the supernatant fractions.

RESULTS

Identification of clone pRED3C6. A total of approximately 3,000 recombinants representing the genome of *S. hyodysenteriae* were screened. One immunopositive recombinant clone, designated pRED3C6, was identified with the mouse monoclonal antibody 10G6/G10. Sequence analysis of the recombinant 2.3-kb DNA fragment in pRED3C6 revealed a single open reading frame encoding a putative protein of approximately 36 kDa (unpublished data).

Specificity of the 2.3-kb DNA fragment. Southern blot hybridization of the [α-^{32}P]dCTP-labeled 2.3-kb fragment from clone pRED3C6 yielded a strong hybridization signal with chromosomal DNA from reference isolates of *S. hyodysenteriae* serotypes 1 through 7 but not with DNA from *S. innocens* B256 and 4/71, WBHIS B359 and B1555a, and *T. succinifaciens* (Fig. 1). When the same probe was reacted with chromosomal DNAs obtained from other cultivable reference isolates of the order *Spirochaetales* as well as enteric bacteria including *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *B. vulgatus* in a slot blot hybridization assay, a specific signal was observed only with chromosomal DNA obtained from reference isolates of *S. hyodysenteriae* serotypes 1 through 9 (data not shown).

PCR and internal probe hybridization. With purified chromosomal DNA from isolates of each of the nine serotypes of *S. hyodysenteriae* as the template, the PCR assay resulted in 1.55-kb products (Fig. 2). The specificity of the 1.55-kb products for *S. hyodysenteriae* was confirmed on the basis of the production of a restriction endonuclease pattern of the PCR products identical to the predicted restriction map analysis of pRED3C6 (data not shown) and a positive hybridization signal with the *S. hyodysenteriae*-specific internal oligonucleotide probe (Fig. 2). The absence of products and a hybridization

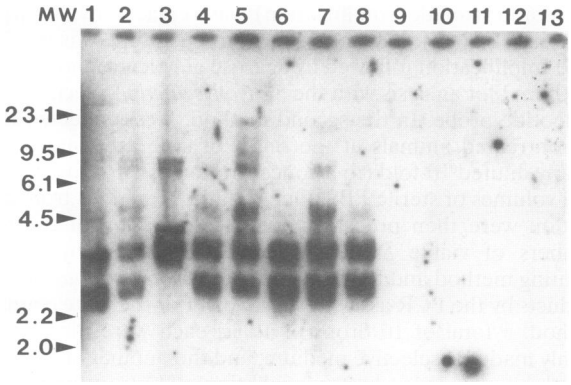

FIG. 1. Southern blot hybridization of a [α-^{32}P]dCTP-labeled 2.3-kb fragment of clone pRED3C6 to purified chromosomal DNA. Purified chromosomal DNA was digested with *Hin*dIII, electrophoresed on a 0.8% agarose gel, and transferred to a nylon membrane. Lanes 1 to 8, *S. hyodysenteriae* B78, B234, B204, B169, A1, B8044, B6933, and AcK 300/8, respectively; lanes 9 to 12, WBHIS B256, B359, B1555a, and 4/71, respectively; lane 13, *T. succinifaciens*.

signal, respectively, after gel electrophoresis and Southern blot hybridization with an internal *S. hyodysenteriae*-specific oligonucleotide probe of PCR-amplified chromosomal DNA obtained from other cultivable reference isolates of the order *Spirochaetales*, including *S. innocens* B256 and 4/71 and other genotypic groups of WBHIS distinct from *S. innocens* as well as enteric bacteria including *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *B. vulgatus*, further confirmed the specificity of the reaction for *S. hyodysenteriae* (data not shown).

PCR detection of *S. hyodysenteriae* in porcine feces. The sensitivities of the PCR assay for detection of *S. hyodysenteriae* in serial 10-fold dilutions of spirochete broth cultures added to normal porcine feces were 1 organism per 0.1 g of feces in the first experiment (data not shown) and 10 organisms per 0.1 g of

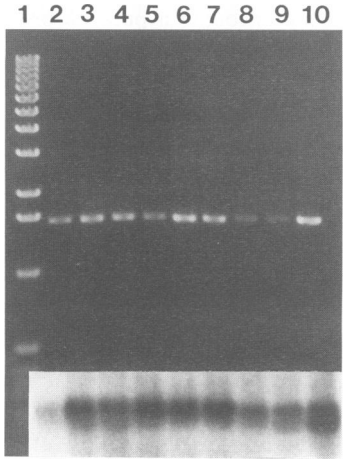

FIG. 2. PCR of purified chromosomal DNA obtained from reference isolates of *S. hyodysenteriae* serotypes 1 through 9. The PCR products were electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. (Inset) Hybridization of transferred DNA from the same gel with an internal *S. hyodysenteriae*-specific oligonucleotide probe 5′ end labeled with [γ-^{32}P]ATP. Lanes: 1, molecular size standard (1-kb DNA ladder; GIBCO-BRL); 2, B78; 3, B204; 4, B169; 5, A1; 6, B8044; 7, B6933; 8, AcK 300/8; 9, FM-88-90; 10, FMV 89-3323.

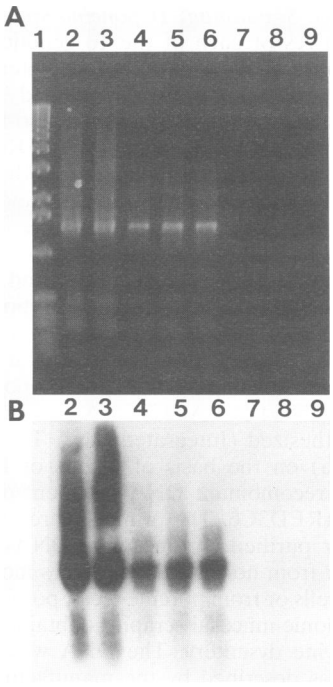

FIG. 3. PCR of purified DNA obtained from porcine feces inoculated with serial 10-fold dilutions of *S. hyodysenteriae* B204 cells. (A) PCR products electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. (B) Transferred DNA from the gel in panel A hybridized with an internal *S. hyodysenteriae*-specific oligonucleotide probe 5′ end labeled with [γ-^{32}P]ATP. Lanes: 1, molecular size standard (1-kb DNA ladder; GIBCO-BRL); lanes 2 to 9, feces containing 10^5, 10^4, 10^3, 10^2, 10^1, 10^0, 10^{-1}, and 10^{-2} *S. hyodysenteriae* cells per 0.1 g, respectively.

feces in the second experiment (Fig. 3). The numbers of spirochetes in dysenteric feces were comparable to those reported previously for the BJ selective culture medium, 1×10^5 and 2×10^5 CFU/0.1 ml, respectively (19). The presence of *S. hyodysenteriae*-specific targets at dilutions of up to 10^{-9} in both fecal specimens by PCR assay indicated a 1,000-fold increase in sensitivity compared with that of conventional culture. The 10^{-10} to 10^{-12} dilutions yielded negative results by both methods. PCR amplification of infected porcine feces, porcine rectal swabs, and porcine colonic mucosal scrapings obtained from nine animals on six different farms generated 1.55-kb products in all samples in which *S. hyodysenteriae* was detected by conventional bacteriological culture identification (Table 1). The one sample which was negative by PCR assay yielded WBHIS by culture.

DISCUSSION

Previous detection of *S. hyodysenteriae* has been labor-intensive and lacking in sensitivity and specificity. In this report we present the use of specific DNA primers for the detection of *S. hyodysenteriae* by PCR amplification of a unique DNA sequence directly from diagnostic specimens. Important observations from these data are as follows. (i) All representatives of *S. hyodysenteriae* serotypes 1 through 9 gave a positive signal as determined by gel electrophoresis and restriction enzyme analysis of the products. (ii) The specificity of the PCR-amplified products obtained from reference strains and field isolates was confirmed by hybridization with a probe internal to

TABLE 1. Comparison of the conventional bacteriological culture identification on selective BJ agar medium and PCR assay for detection of *S. hyodysenteriae* in diagnostic specimens

Farm	No. of samples	Sample type	Result	
			Culture	PCR
A	1	Feces	*S. hyodysenteriae*	+
	2	RS[a]	*S. hyodysenteriae*	+
B	1	MS[b]	*S. hyodysenteriae*	+
C	1	MS	*S. hyodysenteriae*	+
D	1	MS	*S. hyodysenteriae*	+
E	2	Feces	*S. hyodysenteriae*	+
F	1	MS	WBHIS	−
Total 6	9			

[a] RS, porcine rectal swab.
[b] MS, porcine colonic mucosal scraping.

the primer pair. (iii) No signal was detected with bacteria other than *S. hyodysenteriae*, including closely related WBHIS and other members of the order *Spirochaetales*. (iv) The sensitivity of the PCR assay was estimated to be between 1 and 10 organisms per 0.1 g of feces, or 1,000 times that of bacteriological culture on selective BJ medium, thus allowing definitive diagnosis of swine dysentery even when the organism is at levels undetectable by culture methods.

Routine detection of *S. hyodysenteriae* by direct culture methods is based on the pattern and intensity of hemolysis on blood agar; however, this characteristic is very similar for WBHIS, including the nonpathogenic *S. innocens*. More recent studies indicate, however, that hemolysis alone might not be a completely reliable indicator of pathogenicity since both weakly beta-hemolytic enteropathogenic spirochetes and strongly beta-hemolytic nonpathogenic spirochetes have been isolated from swine with diarrhea (9, 13, 25, 36). Also, the sensitivity of the direct culture method depends upon the number of organisms present in the sample, which in turn depends on the stage of the infection of the animal at the time of collection. Kunkle and Kinyon (19) reported that the numbers of *S. hyodysenteriae* organisms in porcine colonic contents at the onset of swine dysentery ranged between 2×10^6 and 2×10^{10} CFU/g when the organisms were cultured with the selective BJ medium. In contrast, subclinically affected animals may shed recoverable numbers of spirochetes only sporadically and in much lower numbers than animals with clinical swine dysentery, often resulting in false-negative culture results. Animals with field cases of swine dysentery also may contain drug residues that adversely affect recovery of viable *S. hyodysenteriae* by culture. Factors which affect the results of routine bacteriological culture are not critical to detection of *S. hyodysenteriae* by PCR.

Several biochemical tests for rapid differentiation of enteropathogenic and nonpathogenic intestinal spirochetes of swine have been proposed (1, 2, 12, 32). Although these biochemical characteristics are highly conserved among field isolates of *S. hyodysenteriae*, WBHIS have been shown to yield highly variable results (1–3, 17, 23, 29, 30, 39). Other methods of differentiating *S. hyodysenteriae* from WBHIS include growth inhibition by discs soaked in antiserum (21) and rapid slide agglutination (3). In addition to problems of nonspecific clumping of spirochetes in the saline control in the slide agglutination test, these tests require large numbers of pure cultures of spirochetes, which can take up to 3 weeks to grow (24). Preabsorption of reference polyclonal antisera with WBHIS increases the specificity of the serological tests; however, occasional *S. hyodysenteriae* isolates continue to be falsely classified as nonpathogenic in these tests. An alternative method using microscopic agglutination under phase-contrast or dark-field microscopy was proposed recently; however, some isolates of *S. hyodysenteriae* gave weaker reactions with this assay than with the slide agglutination test (24). Finally, mouse monoclonal antibodies capable of differentiating *S. hyodysenteriae* from porcine WBHIS have been proposed as potential diagnostic reagents (31, 38). Other studies, however, have indicated that spirochetes other than *S. hyodysenteriae* express antigenic determinants recognized by these reagents and cause false-positive results (37). The fact that no serological reagents are available commercially also limits the applicability of serological techniques to routine diagnosis of swine dysentery.

Recombinant DNA technology has been used to generate oligodeoxynucleotide probes specific for the 16S rRNA of *S. hyodysenteriae*, but the sensitivity of the probe method for detection of spirochetes in feces was equivalent to that of routine bacteriological culture: 10^5 organisms per g of feces (14). Further studies also have suggested that the 16S rRNA probe might not be specific only to *S. hyodysenteriae* (39). Dot blot hybridization with whole chromosomal probes and DNA probes for identification of *S. hyodysenteriae* has been reported (5, 33). Although the sensitivity of the whole chromosomal probes was not reported, colony dot blot hybridization with DNA probes was shown to be only slightly better than culture (10^4 organisms per g of feces). These tests are labor-intensive, require specialized equipment, and have turnaround times that would slow routine laboratory diagnosis.

PCR assay of fecal specimens provides a more rapid and accurate method of obtaining a definitive diagnosis of swine dysentery than anaerobic culture on selective agar medium, i.e., 1 day for PCR in contrast to 2 to 6 days by culture. Additionally, noninvasive diagnostic procedures, such as PCR amplification of DNA obtained from fecal specimens, do not require euthanasia of sick animals for necropsy or shipping of whole-intestine specimens through courier services. The PCR assay may also be useful as a method of identifying subclinically affected animals in herds maintained by individuals who are attempting eradication of swine dysentery without depopulation of the herd (10). Investigations aimed at furthering the understanding of the molecular epidemiology of swine dysentery and the pathogenesis of subclinical infection in swine dysentery are now possible.

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