JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1988, p. 2495–2500
0095-1137/88/122495-06$02.00/0

Vol. 26, No. 12

Repetitive Sequence Element Cloned from *Leptospira interrogans* Serovar Hardjo Type Hardjo-Bovis Provides a Sensitive Diagnostic Probe for Bovine Leptospirosis

RICHARD L. ZUERNER* AND CAROLE A. BOLIN

National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received 31 May 1988/Accepted 25 August 1988

A repetitive sequence element was cloned from the primary etiological agent causing bovine leptospirosis in North America, *Leptospira interrogans* serovar hardjo type hardjo-bovis. This element was used to design a sensitive diagnostic probe which distinguishes hardjo-bovis from other pathogenic leptospires which commonly infect domestic animals in North America and discriminates between hardjo-bovis and the reference strain for serovar hardjo, hardjoprajitno. By using this probe, it was possible to identify infected cattle shedding hardjo-bovis in their urine. This is the first practical demonstration of a cloned DNA probe for leptospirosis, and it provides a sensitive method for studying the transmission and pathogenesis of *L. interrogans* infections. Control measures for *L. interrogans* infections may now be improved by rapidly and efficiently identifying infected animals.

Leptospirosis, caused by *Leptospira interrogans*, is a disease of animals and humans which has a worldwide distribution. *L. interrogans* is an immunologically diverse species and contains several distinct genetic groups (14, 37). In North America, the most common cause of bovine leptospirosis is *L. interrogans* serovar hardjo type hardjo-bovis (8, 32, 36). Hardjo-bovis and the reference strain for serovar hardjo, hardjoprajitno, can be differentiated by restriction endonuclease analysis of genomic DNA (33). However, the existence of similar antigens shared by hardjo-bovis and hardjoprajitno (18) prevents these two bacteria from being differentiated by classical serological techniques.

Infection of cattle with either type of serovar hardjo causes abortions, stillbirths, production of weak offspring, and infertility (4, 5, 7, 31). In addition, cattle infected with serovar hardjo develop persistent renal infections and shed leptospires in their urine (5, 31). Exposure to urine containing hardjo-bovis is considered to be the primary source of infections within herds (23).

Diagnosis of leptospirosis usually depends upon demonstration of serum antibodies. The serologic method of choice is the microscopic agglutination test (2). However, interpretation of microscopic agglutination test results is often subjective and is complicated by numerous factors, including previous vaccination or infection and antigenic heterogeneity among *L. interrogans*. Since cattle infected with hardjo-bovis may fail to produce detectable antibodies (6, 32), an accurate diagnosis of infection with hardjo-bovis requires direct demonstration of *L. interrogans* in tissues, blood, or urine. This is achieved either by bacteriological culture or by immunological techniques. Isolation of serovar hardjo from clinical specimens is labor intensive and inconsistent and requires weeks or months before results are obtained. Similarly, antigens may be degraded or blocked in some clinical specimens and thus prevent immunological detection of bacteria.

Several investigators have utilized DNA-DNA hybridization methods for rapid and reliable detection of *L. interrogans* in biological samples (blood, urine, and tissue homogenates) (24, 29, 30). The probes for these hybridizations consist of genomic DNA labeled by nick translation with radiolabeled or biotinylated nucleotides. Although these probes are specific for *L. interrogans*, they demonstrate extensive cross-hybridization among pathogenic serovars (30).

We have cloned a repetitive sequence element from hardjo-bovis and used this cloned fragment to develop a sensitive diagnostic probe for the detection of hardjo-bovis shed in the urine of infected cattle. This probe distinguishes hardjo-bovis from other pathogenic leptospires which commonly infect domestic animals in North America.

(This work was presented in part at the 38th Annual Meeting of the American Society of Microbiology [R. L. Zuerner and C. A. Bolin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D-66, p. 82].)

MATERIALS AND METHODS

Materials. Restriction endonucleases, plasmid pUC19, phage T3 RNA polymerase, nick translation kit, and molecular weight standards were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Additional restriction endonucleases and phage T4 DNA ligase were purchased from New England BioLabs, Inc., Beverly, Mass. Plasmid pBSM13 was purchased from Stratagene Corp., La Jolla, Calif. Molecular biology-grade calf alkaline phosphatase was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Ion-exchange membrane NA-45 was purchased from Schleicher & Schuell, Inc., Keene, N.H. Hybond-N was purchased from Amersham Corp., Arlington Heights, Ill. Radiolabeled [α-^{32}P]UTP (650 Ci/mmol, 12 mCi/ml) and [α-^{32}P]dGTP (600 Ci/mmol, 12 mCi/ml) were purchased from ICN Pharmaceuticals, Inc., Irvine, Calif.

Bacterial strains. Bacterial strains used in this study are listed in Table 1. *Leptospira* spp. were propagated in bovine serum albumin-Tween 80 medium at 30°C (15). *Escherichia coli* was propagated in double yeast tryptone medium at 37°C (22).

DNA extraction and cloning techniques. Genomic DNA

* Corresponding author.

TABLE 1. Bacterial strains used in this study[a]

Organism and serogroup	Serovar	Type and/or strain	Source[b]
L. interrogans			
Sejroe	Hardjo	Hardjo-bovis 93U	NADC
	Hardjo	Hardjoprajitno	NADC
Canicola	Portland-vere	Lt63-69	NADC
Grippotyphosa	Grippotyphosa	RM-52	NADC
Icterohaemorrhagiae	Copenhageni	M20	NADC
Pomona	Pomona	Kennewicki RM211	NADC
L. biflexa			
Semaranga	Patoc	Patoc I	N. Charon

[a] *E. coli* JM107 Δ(*lac pro*) *thi strA supE endA sbcB hsdR* F′ *traD36 proAB lacI*^{q} Δ*Z* M15 was obtained from J. Neill of the National Animal Disease Center, Ames, Iowa.

[b] NADC strains are from the National Leptospirosis Reference Laboratory, National Animal Disease Center, Ames, Iowa. N. Charon is from West Virginia University, Morgantown.

was extracted from *L. interrogans* as described by Thiermann et al. (34). Plasmid DNA was prepared from *E. coli* cells in 1.5- or 50-ml overnight cultures by using an alkaline lysis method (12).

L. interrogans serovar hardjo type hardjo-bovis DNA was digested with *Nar*I, and the digestion products were separated by agarose gel electrophoresis. Restriction fragments approximately 1.4 kilobases (kb) in length were electrophoretically transferred to an NA-45 membrane. Isolated DNA fragments were then eluted and concentrated by ethanol precipitation (3). The isolated DNA fragments were ligated to *Nar*I-*Acc*I-digested, calf alkaline phosphatase-treated pUC19 and used to transform *E. coli* JM107 to ampicillin resistance (Ap^r). Transformants harboring recombinant plasmids were identified by inactivation of the vector-encoded *lacZ* gene by using the chromogenic lactose analog 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (22). Plasmid DNA was extracted from Lac$^-$ transformants and examined by restriction endonuclease and DNA blot analysis.

Gel electrophoresis and DNA blot analysis. Restriction endonuclease-digested DNA was fractionated by electrophoresis overnight at 50 V in 0.7 or 1% agarose gels buffered with 89 mM Tris–89 mM boric acid–2 mM EDTA (19). Restriction fragments were transferred to Hybond-N (19), and the immobilized DNA was hybridized with probes radiolabeled either with [α-^{32}P]dGTP by nick translation (25) or with [α-^{32}P]UTP by runoff transcription with phage T3 RNA polymerase (21). After hybridization, filters were washed with 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 61°C and used to expose AR film (Eastman Kodak Co., Rochester, N.Y.) at −80°C.

Slot blot analysis of cultured bacteria and urine samples was performed by using a modification of the procedure described by Millar et al. (24). Briefly, the cell concentration of cultured bacteria was determined with a Petroff-Hauser counting chamber by using dark-field microscopy. Cell concentrations were adjusted to 5×10^7 cells per ml with phosphate-buffered saline and serially diluted, by using twofold dilutions, to 2.5×10^4 cells per ml. Samples (100 μl) of these diluted cell suspensions were mixed with 100 μl of 0.5 M NaOH–1.5 M NaCl and incubated for 1 h at room temperature. These suspensions were neutralized with 200 μl of 1 M Tris hydrochloride–1.5 M NaCl, pH 8.0, and 40 μl of the suspension was applied to Hybond-N by using a slot blot apparatus (Schleicher & Schuell).

Urine samples were obtained from cows which were either naturally or experimentally infected with hardjo-bovis (Bolin et al., submitted for publication). Bacteria were concentrated from 1- and 10-ml volumes of urine by centrifugation at 11,000 × *g* for 5 min in a microcentrifuge, suspended in 100 μl of phosphate-buffered saline, and treated as described for cultured cells. Four hundred microliters of these samples was applied to the nylon membranes. After hybridization with radiolabeled probes, the nylon membranes were washed with 2× SSC–0.1% sodium dodecyl sulfate at 61°C and then used to expose AR film (Eastman Kodak) at −80°C. Autoradiographic images were quantitated by scanning the autoradiographs with a laser densitometer (UltraScan XL; LKB Instruments, Inc., Rockville, Md.) and subsequent analysis with Gelscan XL software (LKB Instruments).

RESULTS

Cloning a repetitive sequence element from *L. interrogans* serovar hardjo type hardjo-bovis. Restriction endonuclease analysis of *L. interrogans* serovar hardjo type hardjo-bovis DNA revealed a 1.4-kb *Nar*I fragment which is present at a high copy number within the hardjo-bovis genome (Fig. 1). Similarly, two *Hha*I fragments with sizes of 0.3 and 1.1 kb are also present with approximately the same copy number as the 1.4-kb *Nar*I fragment. These two *Hha*I fragments are thought to compose the 1.4-kb *Nar*I fragment and could arise as a result of similar recognition sequences between these two enzymes; *Nar*I recognizes the sequence GGCGCC, and *Hha*I recognizes GCGC (26). The 1.4-kb *Nar*I fragment was cloned in the plasmid vector pUC19, and the resulting plasmid, designated pLI16, was used for further study.

Restriction enzyme analysis and Southern blot analysis. A restriction enzyme map of pLI16 was constructed (Fig. 2). Unique sites for *Eco*RI, *Hin*dIII, *Cla*I, *Hha*I, *Xmn*I, and *Eco*RV were located within the cloned fragment. The 1-kb *Hin*dIII fragment from pLI16, containing hardjo-bovis DNA and adjacent vector sequences, was subcloned into the RNA transcription vector pBSM13. In the resulting plasmid, pLI17, the insert DNA is flanked by promoters for phage T3 and T7 RNA polymerases (Fig. 3) which can be used to synthesize radiolabeled single-stranded RNA (ssRNA) probes complementary to a portion of the 1.4-kb *Nar*I fragment.

Samples of genomic DNA from *L. interrogans* serovars which commonly infect domestic animals in North America were digested with *Hha*I, *Eco*RI, or *Nar*I, separated by agarose gel electrophoresis, and blotted onto nylon membranes. Although there is no evidence that *L. interrogans* serovar hardjo type hardjoprajitno infects domestic animals

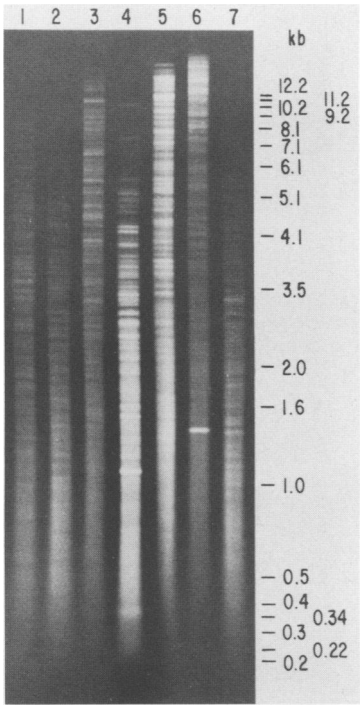

FIG. 1. Restriction endonuclease analysis of hardjo-bovis DNA. Genomic DNA (2.5 μg) purified from *L. interrogans* serovar hardjo type hardjo-bovis 93U was digested with *Cla*I (lane 1), *Eco*RI (lane 2), *Eco*RV (lane 3), *Hha*I (lane 4), *Hin*dIII (lane 5), *Nar*I (lane 6), and *Xmn*I (lane 7). Digestion products were separated in a 1% agarose gel, stained with ethidium bromide, and visualized by UV illumination.

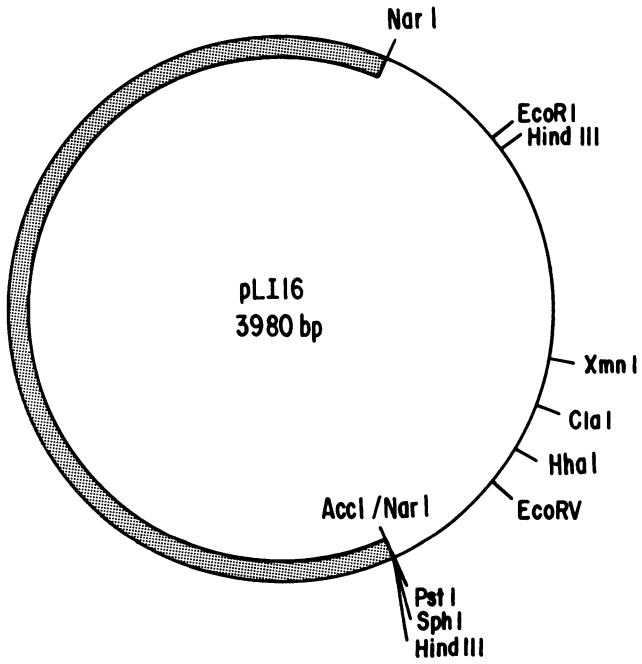

FIG. 2. Restriction enzyme map of pLI16. The 1.4-kb *Nar*I fragment of hardjo-bovis was cloned into the *Nar*I and *Acc*I sites of pUC19. The thick portion represents vector DNA, whereas the thin portion represents cloned DNA.

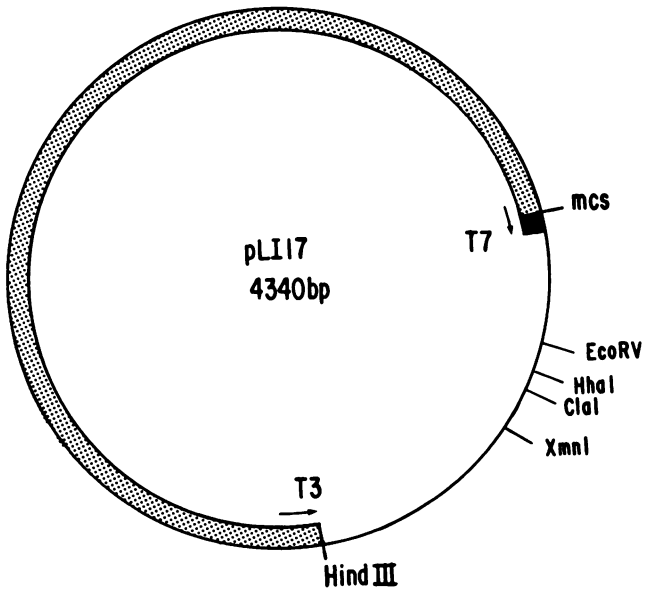

FIG. 3. Restriction enzyme map of pLI17. The 1.0-kb *Hin*dIII fragment of pLI17 was subcloned into pBSM13. The thick portion represents vector DNA, whereas the thin portion represents cloned DNA. The locations and orientations of T3 and T7 promoters are shown. The multiple cloning site (mcs) contains restriction sites (from vector sequences to cloned fragment) for *Eco*RI, *Sac*I, *Kpn*I, *Sma*I, *Bam*HI, *Xba*I, *Acc*I, *Pst*I, *Sph*I, *Hin*dIII, *Sph*I, *Pst*I, and *Acc*I. The last three restriction sites represent the portion of pUC19 vector sequences subcloned from pLI16.

in North America, hardjoprajitno was included in these analyses since it is the reference strain for *L. interrogans* serovar hardjo (33) and is indistinguishable from hardjo-bovis by classical serological techniques. The immobilized DNA was used to hybridize radiolabeled ssRNA transcripts synthesized from *Sac*I-linearized pLI17 by using phage T3 RNA polymerase and [α-^{32}P]UTP. The results (Fig. 4) demonstrate the repetitive nature of this 1.4-kb *Nar*I fragment within the genome and that the 0.3- and 1.1-kb *Hha*I fragments are homologous to the cloned repetitive element. These results also demonstrate the presence of little detectable hybridization between this probe and any of the *L. interrogans* strains tested except serovar hardjo type hardjo-bovis.

Sensitivity and testing of the repetitive sequence probe. Previously described probes developed for diagnosis of *L. interrogans* in biological materials have consisted of genomic DNA labeled with ^{32}P-labeled nucleotides by nick translation (24, 30). Therefore, we compared the sensitivity of a radiolabeled ssRNA probe synthesized from pLI17 with that of a radiolabeled genomic DNA probe. Cultured *L. interrogans* and *L. biflexa* were serially diluted from 5×10^7 cells per ml to 2.5×10^4 cells per ml and lysed. The DNA was denatured, and a portion of these suspensions was filtered through a nylon membrane. The immobilized DNA was used to hybridize either the pLI17-derived radiolabeled ssRNA probe (prepared as described above) or serovar hardjo type hardjo-bovis genomic DNA radiolabeled by nick translation. Both of these probes were radiolabeled to specific activities of approximately 10^9 dpm/μg of nucleic acid. The resulting autoradiographs (Fig. 5) demonstrate that the pLI17-generated probe can detect as few as 1×10^3 hardjo-bovis cells, while the detection limit for the radiolabeled genomic DNA probe was approximately 4×10^3 cells. The

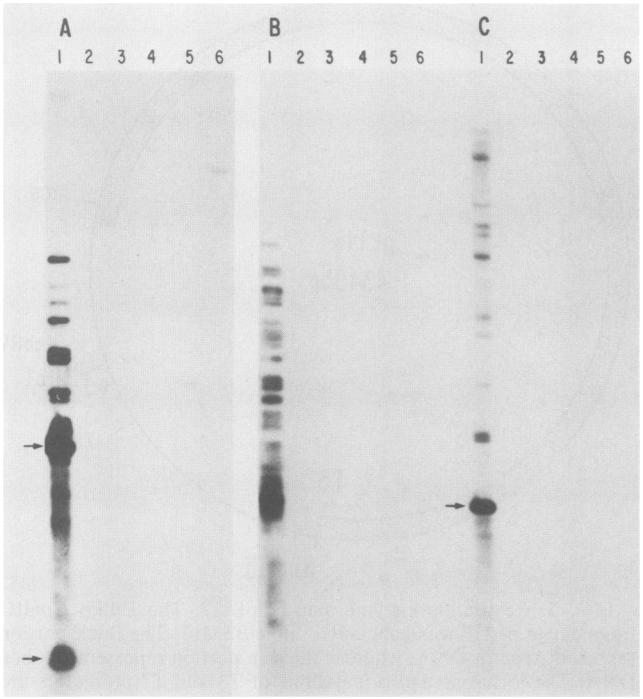

FIG. 4. Southern blot analysis of *L. interrogans* DNA. Genomic DNA (2.5 μg) from *L. interrogans* serovar hardjo type hardjo-bovis (lanes 1), serovar hardjo type hardjoprajitno (lanes 2), serovar pomona type kennewicki (lanes 3), serovar grippotyphosa (lanes 4), serovar copenhageni (lanes 5), and serovar portland-vere (lanes 6) was digested with *Hha*I (A), *Eco*RI (B), and *Nar*I (C). DNA was electrophoresed in 1% (A) or 0.7% (B and C) agarose gels and then blotted onto a nylon membrane and used to hybridize ^{32}P-labeled ssRNA probes synthesized from pLI17. The resulting autoradiographs are shown. Arrows indicate the repetitive sequence fragments detected in ethidium bromide-stained gels and correspond to fragments 0.3 and 1.1 kb (A) or 1.4 kb (C) in length.

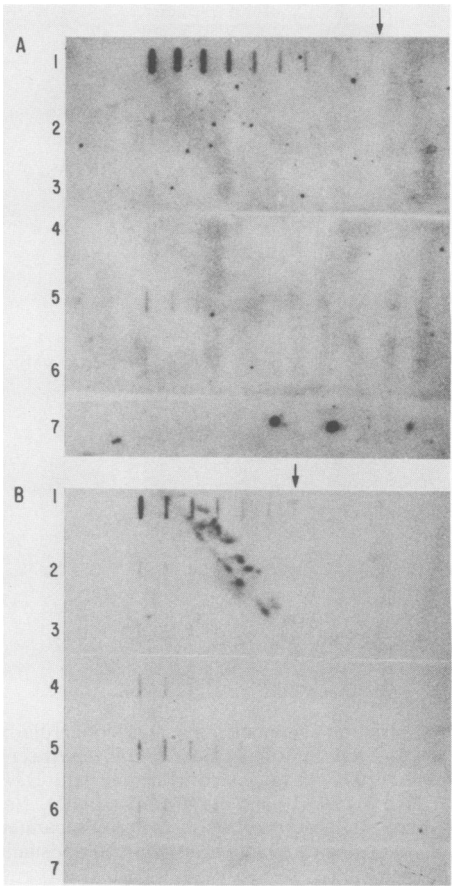

FIG. 5. Comparison of cloned and genomic hardjo-bovis probes. Cultured *L. interrogans* were diluted by using serial doubling dilutions from 5×10^7 cells per ml to 2.5×10^4 cells per ml. The cells were lysed, the DNA was denatured, and the equivalent of 10 μl of the original cell suspensions was applied to nylon membranes. The immobilized DNA was used to hybridize either a ^{32}P-labeled ssRNA probe synthesized from pLI17 (A) or a ^{32}P-labeled hardjo-bovis genomic DNA probe (B). The resulting autoradiographs are shown. Lanes: 1, serovar hardjo type hardjo-bovis; 2, serovar hardjo type hardjoprajitno; 3, serovar pomona type kennewicki; 4, serovar grippotyphosa; 5, serovar copenhageni; 6, serovar portland-vere; 7, *L. biflexa* serovar patoc. Arrows indicate highest dilution of cells detectable by each probe (serial dilutions are in order from lowest [left] to highest [right]).

level of specificity of these two probes for hardjo-bovis was assessed by quantitating autoradiographic signals by scanning laser densitometry and then comparing the values obtained in heterologous reactions with those obtained in homologous reactions. The results of this analysis (Table 2) indicate that the pLI17-derived probe is more specific for hardjo-bovis than the genomic hardjo-bovis DNA probe. Both probes were species specific, as there was no detectable cross-hybridization between either probe and the saprophyte *L. biflexa* serovar patoc.

Transmission of leptospirosis in cattle herds is thought to occur primarily by exposure of animals to urine from infected animals (23). Therefore, the pLI17-derived probe was tested for its ability to detect hardjo-bovis shed in the urine of infected cattle. Bacteria were concentrated from cattle urine and subjected to blot analysis as described above. In this experiment, the negative-control sample was a composite from nine animals collected prior to experimental infection (Bolin et al., submitted). Positive urine samples were collected from experimentally infected animals demonstrating no clinical signs of disease 4, 8, and 12 weeks after exposure to hardjo-bovis (Bolin et al., submitted) and from a naturally infected cow. Slot blots of urine samples and standards of diluted hardjo-bovis cells were hybridized and processed simultaneously with pLI17-derived probes. The results of this experiment are summarized in Table 3 and

TABLE 2. Quantitative comparison of pLI17 and genomic DNA probe specificities

Organism and serovar[a]	% Hybridization[b] with DNA probe from:	
	pLI17	Hardjo-bovis
L. interrogans		
Hardjo type hardjo-bovis	100	100
Hardjo type hardjoprajitno	8	11
Pomona type kennewicki	2	5
Grippotyphosa	5	16
Portland-vere	1	27
Copenhageni	1	11
L. biflexa		
Patoc	<1	<1

[a] Samples in Fig. 5 containing 2.5×10^5 cells.
[b] Comparison of autoradiographic signals with signals obtained with hardjo-bovis.

demonstrate that the pLI17-derived probe detects hardjo-bovis shed in urine from infected animals. Autoradiographic signals were quantitated by scanning laser densitometry and compared with the signals obtained with samples of cultured hardjo-bovis cells. The number of leptospires detected in the urine of infected animals ranged from $<1 \times 10^2$ cells per ml to approximately 3×10^4 cells per ml. As noted (Table 3), most but not all of the urine samples tested were found to contain hardjo-bovis either by culture or by fluorescent antibody (FA) (Bolin et al., submitted). Additionally, culture and FA results of urine samples from cow 86 taken at other times during the infection were positive, thus confirming that this cow was infected and shedding *L. interrogans* in its urine.

DISCUSSION

This report describes the identification and cloning of a discrete repetitive sequence element from *L. interrogans* serovar hardjo type hardjo-bovis. This cloned repetitive element was used to develop a sensitive probe for the diagnosis of bovine leptospirosis. This is the first reported test of clinical material using cloned *L. interrogans* DNA to determine the practicality of such probes for identifying hardjo-bovis-infected cattle.

Repetitive sequence DNA is attractive for the development of diagnostic probes, since the target sequences for probes are amplified naturally within the genome. This attribute enables detection of fewer organisms with probes directed to repetitive sequence elements compared with probes directed to single-copy sequences. Repetitive sequence DNA has been used to develop diagnostic probes for eucaryotic parasites *Plasmodium falciparum* (10) and *Brugia malayi* (28). Since repetitive sequence DNA is a common feature of eucaryotic (13) but not procaryotic genomes, few diagnostic probes for pathogenic bacteria utilize repetitive sequence DNA. Instead, probes to unique sequences contained within bacterial rRNA have been developed, since the target sequences for these probes are amplified as a result of ribosome biosynthesis (11, 16). Our levels of detection with the repetitive sequence probe compare favorably with those from reports describing rRNA probes developed for detecting other bacteria (11, 16).

Restriction endonuclease analysis of genomic DNA demonstrated the presence of the 1.4-kb *Nar*I fragment at a high copy number within the *L. interrogans* serovar hardjo type hardjo-bovis genome. While eucaryotic repetitive sequence elements have been demonstrated by using similar methods (1), previous descriptions of repetitive sequence DNA in bacteria have relied upon cloning random segments from the genome and subsequently demonstrating that such fragments are repetitive by Southern blot analysis (9, 27). The high copy number of this fragment, estimated to be approximately 40 copies per genome, may be one factor which enables such easy detection of the repetitive fragment.

TABLE 3. Test of pLI17 probe with cattle urine samples

Sample	Time postinfection (mo)[a]	Concn (cells/ml)[b]	Culture[c]	FA[d]
Negative control		$<1 \times 10^2$	−	−
Cow 103	1	3×10^2	+	+
	2	4×10^3	−	+
	3	1×10^2	+	+
Cow 79	2	7×10^3	+	+
	3	2×10^3	−	+
Cow 86	2	4×10^2	−	−
	3	1×10^2	−	−
Cow 80	2	3×10^4	+	+
40U		4×10^3	+	+

[a] Time following experimental infection of cattle with hardjo-bovis. 40U was naturally infected, and time of exposure could not be determined.
[b] Bacterial cell concentrations in urine normalized to cells per milliliter as determined with pLI17.
[c] Results of attempts to culture urine samples (Bolin et al., submitted).
[d] Results of FA with anti-hardjo-bovis conjugate (Bolin et al., submitted).

Reports describing cloned *L. interrogans* probes have not included tests to determine their sensitivity (16, 35). In order for such probes to provide a viable alternative to existing diagnostic techniques, it is imperative that the number of cells such probes can detect be determined and that these values be relevant to the levels of bacteria shed by infected animals. The probe described in this report can detect as few as 1×10^3 cells, which, when used by us, was approximately fourfold more sensitive than a genomic DNA probe. One previously described genomic DNA probe reportedly detects approximately 1×10^3 cells, and therefore may be of equal sensitivity to the pLI17-derived probe (24). However, genomic DNA probes demonstrate significant cross-hybridization with other serovars of *L. interrogans* (30). Probes derived from the hardjo-bovis repetitive sequence element demonstrated little detectable hybridization with any of the serovars commonly isolated from domestic animals in North America (serovars grippotyphosa, hardjo, copenhageni, pomona, and portland-vere) except for hardjo-bovis. Likewise, little cross-hybridization was observed between this probe and hardjoprajitno, the reference strain for serovar hardjo. The level of cross-hybridization which was detected between the pLI17-derived probe and DNA from other serovars was titrated out before clinically relevant levels of bacteria were reached and should not affect the use of these probes for diagnosis of hardjo-bovis infections.

The pLI17-derived probe is sensitive enough to detect hardjo-bovis shed in the urine of cattle and thus offers an effective method to rapidly identify potential sources of hardjo-bovis infections within herds. Indeed, this probe detected hardjo-bovis in each of the urine samples tested, whereas culture and FA results varied. These findings are most easily explained by examining the requirements for each of these techniques. Bacteriological culture has an absolute requirement for obtaining viable organisms. Viable cells are likely to constitute a fraction of the total number of cells in a given sample, and this fraction is influenced by collection, transport, and storage conditions prior to inoculation of media. Similarly, positive FA results require access of antibodies to antigens. Negative FA results may occur as a result of coating of the bacteria with host proteins (e.g., antibodies) or by degradation of surface antigens. In contrast, nucleic acid probes only require the presence of target DNA sequences, some of which may be degraded.

The sensitivity and specificity of the pLI17-derived probe should make possible preliminary diagnoses of hardjo-bovis infections on the basis of positive urine samples. Previously such determinations have only been possible through bacteriological culturing (which often requires several weeks or months for primary isolation) followed by restriction endonuclease analysis (20, 34), microscopic agglutination test (2), or DNA blot analysis (17, 35). This probe offers a more rapid diagnosis for bovine leptospirosis than techniques currently in use. Incorporation of nonisotopic labeling methods with this probe should enable veterinary laboratories to efficiently perform large-scale herd screening for leptospirosis and thus offer livestock producers a tool for better herd health man-

agement. Furthermore, the ability of this probe to quantitate the number of *L. interrogans* shed in urine should provide a useful research tool in understanding factors affecting transmission and pathogenesis of leptospirosis.

ACKNOWLEDGMENTS

We thank Annette Handsaker and John Foley for technical assistance and Paul Kapke, Julia Ridpath, and J. Glenn Songer for reviewing the manuscript.

LITERATURE CITED

1. **Botchan, M. R.** 1974. Bovine satellite I DNA consists of repetitive units 1,400 base pairs in length. Nature (London) **251:** 288–292.
2. **Cole, J. R., Jr., C. R. Sulzer, and A. R. Pursell.** 1973. Improved microtechnique for the leptospiral microscopic agglutination test. Appl. Microbiol. **25:**976–980.
3. **Dretzen, G., M. Bellard, P. Sassone-Corsi, and P. Chambon.** 1981. A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112:**295–298.
4. **Ellis, W. A., J. J. O'Brien, D. G. Bryson, and D. P. Mackie.** 1985. Bovine leptospirosis: some clinical features of serovar *hardjo* infection. Vet. Rec. **117:**101–104.
5. **Ellis, W. A., J. J. O'Brien, S. D. Neill, and D. G. Bryson.** 1986. Bovine leptospirosis: experimental serovar *hardjo* infection. Vet. Microbiol. **11:**293–299.
6. **Ellis, W. A., J. J. O'Brien, S. D. Neill, and J. Hanna.** 1982. Bovine leptospirosis: serological findings in aborting cows. Vet. Rec. **110:**178–180.
7. **Ellis, W. A., J. J. O'Brien, S. D. Neill, J. Hanna, and D. G. Bryson.** 1976. The isolation of a leptospire from an aborted bovine fetus. Vet. Rec. **99:**458–459.
8. **Ellis, W. A., and A. B. Thiermann.** 1986. Isolation of leptospires from the genital tract of Iowa cows. Am. J. Vet. Res. **47:**1694–1696.
9. **Flores, M., V. Gonzalez, S. Brom, E. Martinez, D. Pinero, D. Romero, G. Davila, and R. Palacios.** 1987. Reiterated DNA sequences in *Rhizobium* and *Agrobacterium* spp. J. Bacteriol. **169:**5782–5788.
10. **Franzen, L., G. Westin, R. Shabo, L. Aslund, H. Perlman, T. Perrson, H. Wigzell, and U. Petterssonn.** 1984. Analysis of clinical specimens by hybridization with probe containing repetitive DNA from *Plasmodium falciparum*: a novel approach to malaria diagnosis. Lancet **i:**525–527.
11. **Gobel, U. B., A. Geiser, and E. J. Stanbridge.** 1987. Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between *Mycoplasma* species. J. Gen. Microbiol. **133:**1969–1974.
12. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9:**2989–2998.
13. **Jelinek, W. R., and C. W. Schmid.** 1982. Repetitive sequences in eukaryotic DNA and their expression. Annu. Rev. Biochem. **51:** 813–844.
14. **Johnson, R. C., and S. Faine.** 1984. Genus I. *Leptospira* Noguchi 1917, 755^{AL}, p. 62–67. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
15. **Johnson, R. C., and V. G. Harris.** 1967. Differentiation of pathogenic and saprophytic leptospires. I. Growth at low temperatures. J. Bacteriol. **94:**27–31.
16. **Kohne, D., J. Hogan, V. Jonas, E. Dean, and T. H. Adams.** 1986. Novel approach for rapid and sensitive detection of microorganisms: DNA probes to rRNA, p. 100–112. *In* L. Leive (ed.), Microbiology—1986. American Society for Microbiology, Washington, D.C.
17. **Le Febvre, R.** 1987. DNA probe for detection of the *Leptospira interrogans* serovar hardjo genotype hardjo-bovis. J. Clin. Microbiol. **25:**2236–2238.
18. **Le Febvre, R. B., A. B. Thiermann, and J. Foley.** 1987. Genetic and antigenic differences of serologically indistinguishable leptospires of serovar hardjo. J. Clin. Microbiol. **25:**2094–2097.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Marshall, R. B., B. E. Wilton, and A. J. Robinson.** 1981. Identification of leptospira serovars by restriction-endonuclease analysis. J. Med. Microbiol. **14:**163–166.
21. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
22. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101:**20–78.
23. **Michna, S. W.** 1970. Leptospirosis. Vet. Rec. **86:**484–496.
24. **Millar, B. D., R. J. Chappel, and B. Adler.** 1987. Detection of leptospires in biological fluids using DNA hybridization. Vet. Microbiol. **15:**71–78.
25. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:** 237–251.
26. **Roberts, R. J.** 1987. Restriction enzymes and their isoschizomers. Nucleic Acids Res. **15:**R189–R215.
27. **Sapienza, C., and W. F. Doolittle.** 1982. Unusual physical organization of the *Halobacterium* genome. Nature (London) **295:**384–389.
28. **Sim, B. K. L., W. F. Piessens, and D. F. Wirth.** 1986. A DNA probe cloned in *Escherichia coli* for the identification of *Brugia malayi*. Mol. Biochem. Parasitol. **19:**117–123.
29. **Terpstra, W. J., G. J. Schoone, G. S. Ligthart, and J. Ter Schegget.** 1987. Detection of *Leptospira interrogans* in clinical specimens by *in situ* hybridization using biotin-labelled DNA probes. J. Gen. Microbiol. **133:**911–914.
30. **Terpstra, W. J., G. J. Schoone, and J. Ter Schegget.** 1986. Detection of leptospiral DNA by nucleic acid hybridization with ^{32}P- and biotin-labelled probes. J. Med. Microbiol. **22:**23–28.
31. **Thiermann, A. B.** 1982. Experimental leptospiral infections in pregnant cattle with organisms of the Hebdomadis serogroup. Am. J. Vet. Res. **43:**780–784.
32. **Thiermann, A. B.** 1983. Bovine leptospirosis: bacteriologic versus serologic diagnosis of cows at slaughter. Am. J. Vet. Res. **44:**2244–2245.
33. **Thiermann, A. B., A. L. Handsaker, J. W. Foley, F. H. White, and B. F. Kingscote.** 1986. Reclassification of North American leptospiral isolates belonging to serogroups Mini and Sejroe by restriction endonucleases analysis. Am. J. Vet. Res. **47:**61–66.
34. **Thiermann, A. B., A. L. Handsaker, S. L. Moseley, and B. F. Kingscote.** 1985. New method for classification of leptospiral isolates belonging to serogroup Pomona by restriction endonuclease analysis: serovar *kennewicki*. J. Clin. Microbiol. **21:**585–587.
35. **Van Eys, G. J. J. M., J. Zaal, G. J. Schoone, and W. J. Terpstra.** 1988. DNA hybridization with hardjo-bovis specific recombinant probes as a method for type discrimination of *Leptospira interrogans* serovar *hardjo*. J. Gen. Microbiol. **134:**567–574.
36. **White, F. H., K. R. Sulzer, and R. W. Engel.** 1982. Isolations of *Leptospira interrogans* serovars *hardjo*, *balcanica*, and *pomona* from cattle at slaughter. Am. J. Vet. Res. **43:**1172–1173.
37. **Yasuda, P. H., A. G. Steigerwalt, K. R. Sulzer, A. F. Kaufmann, F. Rogers, and D. J. Brenner.** 1987. Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. Int. J. Syst. Bacteriol. **37:**407–415.