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DNA Probe for Detection of the *Leptospira interrogans* Serovar Hardjo Genotype Hardjo-Bovis

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A DNA probe is described for the diagnostic and taxonomic identification of the North American cattle pathogen *Leptospira interrogans* genotype hardjo-bovis. The probe is specific for this genotype and does not hybridize to genomic DNA of any other leptospire pathogen commonly found in North America.

Leptospirosis is an infectious disease found in all parts of the world. The etiologic agent is the spirochete *Leptospira interrogans*, of which there are 19 serogroups and more than 180 serovars. The most common cattle pathogens in North America, Great Britain, New Zealand, Australia, and Argentina are members of the hardjo serovar in the Sejroe serogroup (5, 11, 17). The organisms are pathogens of humans in these regions as well. The disease causes abortion, infertility, agalactia, and premature birth in cattle. It is also common for an infected animal to become a long-term shedder of leptospires, thus perpetuating the disease within a herd.

Animals are protected against leptospirosis by vaccination with chemically or heat-inactivated organisms. Serological analysis of isolates from infected animals is used to identify the pathogenic leptospires endemic to a geographical area. Bacterins are then prepared from these strains. However, in relation to vaccine development and identification of infecting leptospires, identification based on serology poses two major problems: the difficulty of accurate pathogen identification due to the high degree of cross-reactivity between leptospires and the difficulty in distinguishing antibody titers of a chronically infected animal(s) from titers stimulated by vaccination. With bovine leptospirosis, both of these are real problems. Historically, the North American bovine pathogen of the hardjo serovar and the reference strain hardjo prajitno are indistinguishable by serological methods. Thus, virtually all leptospire bacterins contain reference strain hardjo prajitno (unpublished results). However, by means of more-sophisticated methods such as restriction endonuclease analysis (10, 18) and DNA hybridization studies (8), it has been shown that North American cattle are predominantly infected with a leptospire other than hardjo prajitno. This organism has been termed hardjo-bovis. Thus, it is conceivable that the apparent lack of complete protection from a vaccine could be because of infection by hardjo-bovis in a herd vaccinated against hardjo prajitno. To make the distinction between the strains serologically is difficult at best, and thus, the only means of correct identification is the arduous task of culturing strains. It is likely that more specific and more sensitive tools will be needed for identification of infecting leptospires if vaccines more efficacious against leptospirosis are to be developed and if infected animals are to be identified unequivocally. In this report I describe the development of a genetic probe specific for the hardjo-bovis pathogen and intended for the identification of animals infected with this organism, including those previously vaccinated.

Leptospire serovars were obtained from the National Leptospirosis Reference Center located at the National Animal Disease Center, Ames, Iowa. The organisms were grown in bovine serum albumin-polysorbate 80 medium (4) as modified by Johnson and Harris (6). Serogroups, serovars, and isolates used in this study are listed in Table 1.

Genomic DNA was isolated by a previously described procedure (19). Only hardjo prajitno and hardjo-bovis DNA were used in preliminary attempts to identify unique genetic fragments for these organisms. Digestion of the genomes with restriction endonucleases, electrophoresis, Southern blotting (13), and hybridization of leptospiral genomic DNA were performed as described by LeFebvre and Thiermann (8). Study of autoradiographs representing blots probed with either radiolabeled hardjo prajitno or hardjo-bovis DNA revealed several regions in which little to no homology was observed. Corresponding regions of agarose gels were cut out, and the DNA was electroeluted (9). The fragments were ligated into pUC8 plasmids by standard procedures (9) and were used to transform competent *Escherichia coli* JM83 cells. Cells containing recombinant plasmids were selected by growing the cells in the presence of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), a histochemical substrate for β-D-galactosidase in the presence of which Lac^+ colonies or nonrecombinants are blue. White colonies, which contained recombinant plasmids, were subcloned. Recombinant plasmids were isolated by the method of Birnboim (2). The plasmids were radiolabeled by nick translation (12) and were used to probe Southern blots containing *Eco*RI digests of

TABLE 1. Classification of leptospires

Genus and serogroup	Serovar	Reference strain or isolate
Leptospira		
Sejroe	Hardjo	Hardjo prajitno
	Hardjo	Hardjo-bovis A[a]
	Balcanica	IG27 Burgas
Mini	Georgia	117
Pomona	Pomona	Pomona
	Kennewicki	1026
Grippotyphosa	Grippotyphosa	Moskva V
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Leptonema, illini	Illini	3055

[a] Isolate.

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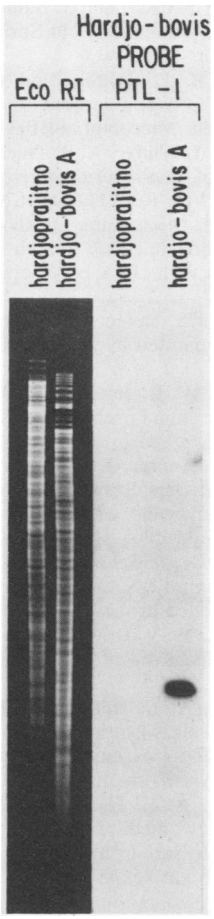

FIG. 1. Electrophoretic and hybridization patterns of *Eco*RI-digested chromosomal DNA from serogroup Sejroe probed with pTL1 (a ^{32}P-labeled DNA probe isolated from hardjo-bovis A). Electrophoresis was performed in a 0.7% agarose gel.

hardjo prajitno and hardjo-bovis genomic DNA. Several recombinant plasmids were identified as being unique to either hardjo prajitno or hardjo-bovis. In this report only one of them, pTL1, will be discussed in detail.

The recombinant pTL1 contains a hardjo-bovis DNA fragment of approximately 4 kilobase pairs. Figure 1 is an autoradiograph of a Southern blot containing approximately 1.5 μg of *Eco*RI-digested hardjo prajitno and hardjo-bovis genomic DNAs probed with approximately 500 ng of radiolabeled pTL1. The hardjo-bovis fragment hybridized only to itself under stringent conditions, with washes of 0.1× sodium chloride-sodium citrate (diluted from a 20× stock of 3M sodium chloride and 0.3 M sodium citrate) and 0.5% sodium dodecyl sulfate at 68°C.

Restriction endonuclease analysis patterns of eight *Leptospira* serovars after digestion with *Eco*RI and an autoradiograph of a Southern blot of the same digestion fragments probed with pTL1 are shown in Fig. 2. The pTL1 probe hybridized only to the hardjo-bovis isolate and slightly to the balcanica reference strain. Previous work with hybridization (8) and guanine-plus-cytosine analysis (unpublished results) has shown hardjo-bovis and the balcanica strain, both of the Sejroe serogroup, to be very similar. However, for local diagnostic purposes, the hybridization of the probe to serovar balcanica does not necessarily pose a problem because this strain is not known to be present in the United States.

Prior unpublished work has shown that pTL1 hybridizes to hardjo-bovis B and C, the other two representatives of North American hardjo isolates. Except for some subtle differences in a few restriction sites, these three subgenotypes are very closely related, as demonstrated by DNA homology studies (8) and guanine-plus-cytosine analysis. Hardjo-bovis A isolates are the most prevalent in North America, which is why their genotype was used for the isolation of the probe.

The pTL1 plasmid probe represents a sensitive, specific, and rapid diagnostic tool that can distinguish between hardjo prajitno and hardjo-bovis. None of the other available methods of diagnosis, including monoclonal antibodies (15), radioimmunoassay (1), chemiluminescence immunoassay (20), enzyme-linked immunosorbent assay (3), microscopic agglutination (3), hemagglutination (14), factor analysis assays (7), and labeling of entire genomic DNA for use as a probe (16), have been able to distinguish between leptospires at this level of specificity.

The development of the genetic probes for pathogenic leptospires described in this report provides, for the first time, a diagnostic tool that may unequivocally identify

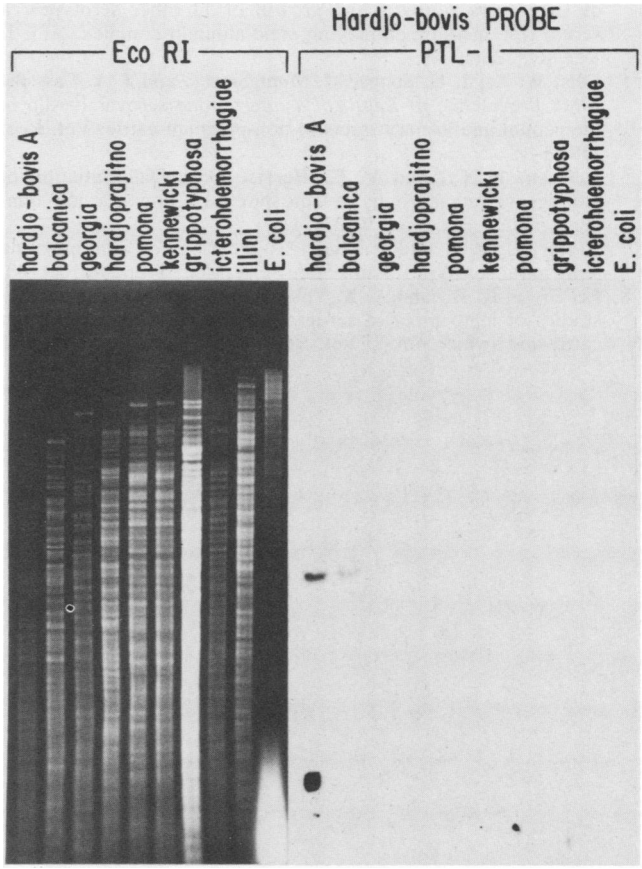

FIG. 2. Electrophoretic and hybridization patterns of *Eco*RI-digested chromosomal DNA from leptospire serogroups and *E. coli* probed with pTL1. Lanes (from left): Hardjo-bovis A from serogroup Sejroe; serovar balcanica from serogroup Sejroe; serovar georgia from serogroup Mini; reference strain hardjo prajitno from serogroup Sejroe; serovar pomona from serogroup Pomona; serovar kennewicki from serogroup Pomona; serovar grippotyphosa from serogroup Grippotyphosa; serovar icterohaemorrhagiae from serogroup Icterchaemorrhagiae; serovar illini from serogroup Illini; and *E. coli*.

animals infected with hardjo-bovis and do so within a matter of hours instead of the days or weeks previous assays required. Genetic probes such as pTL1 may enable researchers, also for the first time, to correctly diagnose infection in animals which have been previously vaccinated. This capability should be very useful in determining the efficacy of current vaccines in providing protection against subsequent infection.

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