Ribosomal DNA Probe for Differentiation of *Babesia microti* and *B. gibsoni* Isolates

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The objective of this study was to determine whether different isolates of *Babesia microti* could be distinguished from morphologically similar isolates of *B. gibsoni* by using a ribosomal DNA (rDNA) probe. A *Babesia*-specific rDNA probe was obtained by polymerase chain reaction amplification of sequences from *B. microti* DNA using universal primers directed against highly conserved portions of the eukaryotic 16S-like rRNA gene. The chemiluminescent rDNA probe hybridized to Southern blots of restriction endonuclease-digested DNA preparations of different isolates of *B. gibsoni* from infected dogs and *B. microti* from infected humans and white-footed mice. Restriction fragment length polymorphisms served to differentiate these species. Although the hybridization patterns seen with DNAs from six *B. microti* isolates did not vary, those of the five *B. gibsoni* isolates did indicate genotypic variation. We concluded that isolates of *B. microti* and *B. gibsoni* can be differentiated on the basis of restriction fragment length polymorphism detected with a chemiluminescent rDNA probe.

Babesia species are intraerythrocytic, tick-transmitted protozoal parasites that infect a variety of mammals, both wild and domestic. Severe, often fatal cases of human babesiosis due to infection with bovine babesial parasites have been reported in Europe (12, 43). In the United States, human babesial infections have been associated primarily with *Babesia microti* in New York and New England, where the parasite is transmitted from white-footed mice (*Peromyscus leucopus*) to humans by *Ixodes dammini* ticks (33, 39, 40). Serological surveys in Cape Cod and Boston, Mass. (31), and Shelter Island, N.Y. (15), have shown that 3.7 to 6.9% of the healthy individuals tested were seropositive for *B. microti* antibodies and hence had been exposed to the parasite. Increasing numbers of clinical and asymptomatic cases of human babesiosis, including many outside of the New England area, are being reported (5, 6, 12, 16, 18, 21, 32, 38, 41).

Despite the importance of babesial parasites of humans and domestic animals, little research has been directed towards their characterization. Identification of babesial species is based mainly on morphology, particularly size, and host specificity (22). *Babesia* species are classified as small (1.5 to 2.5 μm) or large (3 to 5 μm); however, within these groups parasites from different hosts are often markedly similar in morphology. The generalization that *Babesia* species are strictly host specific, traditionally considered to be a clue for species designation, is no longer tenable since the discovery that parasites such as *B. microti* may infect not only a variety of small terrestial mammals (4, 13, 37, 44) but also a variety of subhuman primates (24, 34) and humans. *B. divergens* has similarly been found to infect rats (28), gerbils (26), and humans (12), as well as its main bovine host.

Because the host range for these and other *Babesia* species is probably even wider than is presently recognized, diagnosis of babesial species infecting atypical hosts requires improved methods for parasite identification.

The present study sought to differentiate between two small babesial parasites of clinical significance, *B. microti* and *B. gibsoni*. *B. gibsoni* is a pathogen of dogs in Asia and Egypt which has been identified in the United States (2, 11). Human babesiosis and canine babesiosis exhibit similar clinical manifestations, predominantly fever, anorexia, malaise, and anemia (11, 14, 18, 43). *B. microti* and *B. gibsoni* cannot be reliably distinguished on the basis of light microscopic examination of Giemsa-stained blood smears, and they show considerable antigenic cross-reactivity in the indirect fluorescent-antibody test (42); these are the primary methods for the diagnosis of babesiosis (8). Knowledge of the natural tick vectors and host range of these parasites, particularly in North America, is insufficient for these characteristics to be used as reliable taxonomic criteria. Therefore, we determined whether restriction fragment length polymorphisms (RFLPs), detected by hybridization of a chemiluminescent rDNA probe to endonuclease digests of DNAs from different *B. microti* and *B. gibsoni* isolates, could be used for parasite identification.

MATERIALS AND METHODS

Parasites. *B. gibsoni* parasites were obtained from splenectomized dogs that had been experimentally inoculated with *B. gibsoni*-infected erythrocytes. Three of the dogs received blood from different dogs that had naturally acquired *B. gibsoni* infections in Lancaster (1Bg and 2Bg) and Apple Valley (7Bg), Calif. Dogs 3Bg and 4Bg were each infected by intravenous and subcutaneous inoculations of cryopreserved stabilates containing a total of approximately

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1.4 × 10^7 *B. gibsoni*-infected erythrocytes obtained from dog 2Bg. *B. microti* isolates were obtained from golden Syrian hamsters (100 to 200 g) that were inoculated intraperitoneally with cryopreserved stabilates of *B. microti*; these isolates had in turn been derived from infected humans or white-footed mice (*P. leucopus*) and maintained by serial blood passage through hamsters (4). Isolates 1Bm (GI strain) (30) and 2Bm originated from humans who acquired their infections on Nantucket Island, Mass. The remaining isolates were derived from infected humans (3Bm and 4Bm) residing in Old Lyme and Stonington, Conn., and white-footed mice that were trapped near the homes of infected humans (5Bm and 6Bm) in Stonington, Conn. (16).

rDNA probe. A *Babesia*-specific ribosomal DNA (rDNA) probe was obtained by polymerase chain reaction (PCR) amplification of *B. microti* DNA by using universal primers directed against highly conserved portions of the eukaryotic 16S-like rRNA gene. *B. microti* parasites were concentrated from 100 μl of infected hamster blood by hypotonic lysis in 2 volumes of TE (10 mM Tris [pH 7.4], 1 mM EDTA) (20) and centrifuged at 16,000 × *g* in 0.5-ml microcentrifuge tubes. The pellets were washed three times in TE, taking care to remove the erythrocyte ghosts after each washing. After the final washing, pelleted merozoites and leukocytes were free of visible hemoglobin. Blood processed in an identical manner from an uninoculated hamster was used as a negative control. The pellets were dispersed in 50 μl of K buffer (50 mM Tris [pH 8.3], 1.5 mM MgCl$_2$, 0.5% Nonidet P-40, 0.5% Tween 20, 10 μg of proteinase K [Sigma Chemical Co., St. Louis, Mo.] per ml) by vortexing and then incubated at 55°C for 1 h. After 10 min of incubation at 95°C, 5 μl of the cleared digest was used in a 50-μl PCR reaction containing 50 mM Tris buffer (pH 8.3), 1.5 mM MgCl$_2$, a 200 mM concentration of each of the four deoxynucleoside triphosphates, 0.25 U of *Taq* polymerase (Perkin Elmer Cetus), and a 50 pM concentration each of universal primer A (23) (5' CCG AAT TCG TCG ACA CCT GGT TGA TCC TGC CAG T 3') and primer C (5' GGG CCC TAG GTG GCG CCG ACG ACC GTG GTC TGA ACG GGA G 3'). Amplification was performed by 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 3 min of extension at 72°C for 30 cycles. The 589-bp amplification product was visualized on an ethidium bromide-stained 1.5% agarose gel (Seakem ME; FMC Bioproducts, Rockland, Maine). Under the conditions used, only a weak signal was produced from the control cell pellets, probably because of the greater number of target genomes present in the pellets from the infected hamster. The amplification products from the *B. microti*-containing pellets were then ligated into a TA cloning vector (Invitrogen, La Jolla, Calif.) as recommended by the manufacturer. Clones containing inserts were selected; two such clones were sequenced by using a Sequenase kit (U.S. Biochemical Co., Cleveland, Ohio). The sequence of the clone and its use as a PCR target for detection of *B. microti* are described elsewhere (27). To generate a *B. microti*-specific rDNA probe, PCR amplification of one of the plasmid clones was performed, as described above, by using primers A and C. The amplification product was labeled and used for hybridization as described below.

DNA preparation. Blood was collected from infected animals when >20% of the erythrocytes contained parasites. Plasma and leukocytes were removed from the blood after centrifugation (400 × *g*, 10 min, 4°C), and the erythrocytes were washed once in cold, sterile phosphate-buffered saline. Pelleted erythrocytes were suspended in 10 volumes of

medium M199 with Earle's balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) with 100 U of penicillin per ml and 100 μg of streptomycin sulfate per ml. Twenty-milliliter aliquots of suspended cells were incubated in 75-cm^2 tissue culture flasks at 37°C in 5% CO$_2$-air with 95% humidity to allow merozoites to accumulate in the medium. After 24 h, the cultures were resuspended and erythrocytes, many containing parasites, were pelleted by centrifugation at 700 × *g* for 10 min at 4°C (P1). The supernatant was removed and centrifuged (1,000 × *g*, 10 min, 4°C). Merozoites were obtained for DNA isolation by centrifugation (16,000 × *g*, 10 min, 4°C) of the resultant supernatant (P3). Control mammalian cell DNA was prepared from leukocytes that were separated from uninfected canine, human, and hamster blood by differential centrifugation (400 × *g*, 20 min, 4°C) on Ficoll-Paque (Pharmacia LKB Biotechnology, Piscataway, N.J.) gradients. DNA was prepared from *Babesia*-infected erythrocytes (P1) and leukocytes as described previously for theilerial piroplasms and lymphoblastoid cells (10). The P3 merozoite preparations were processed by the same method but did not require erythrocyte lysis.

Restriction enzyme digestion and analysis of DNA. Restriction endonuclease digests of approximately 1 μg of merozoite or leukocyte DNA were carried out to completion by using a total of 40 to 72 U of either *Eco*RI, *Hin*dIII, *Hae*III, or *Sau*3A (Boehringer Mannheim, Indianapolis, Ind., or Bethesda Research Laboratories, Gaithersburg, Md.) per μg of DNA. Half of the total enzyme volume was incubated with the DNA for 1 to 2 h at 37°C, after which the remaining enzyme was added and incubated for an additional 1 to 2 h at 37°C. DNA fragments were separated by electrophoresis in horizontal gels of either 0.8% (wt/vol) agarose or a combination of 1% (wt/vol) agarose with 2% (wt/vol) NuSieve agarose (FMC Bioproducts) in TBE (45 mM Tris-borate, 1 mM EDTA) at 40 V for 16 to 18 h. Standards representing molecular size fragments were included in at least one lane on each gel (*Hin*dIII digest of lambda DNA and *Hae*III digest of ϕX174 DNA; Bethesda Research Laboratories). Gels were stained with ethidium bromide (0.5 μg ml^{-1}) and photographed under UV illumination. Restriction fragments were denatured in the gel and transferred onto nylon filters (Hybond NT; Amersham Corporation, Arlington Heights, Ill.) by Southern blotting as previously described (35). DNA was cross-linked to the nylon filters by using a Stratalinker UV Crosslinker (Stratagene, La Jolla, Calif.).

Prehybridization, preparation of the labelled probe, and hybridization were performed as recommended by the manufacturer for the enhanced chemiluminescence gene detection system (Amersham). Labelled probe was added to a final concentration of 10 ng/ml of hybridization solution and incubated overnight at 37°C with gentle agitation. After hybridization, the filters were washed twice for 20 min each at 38°C in the enhanced chemiluminescence primary wash buffer containing either 0.1× or 2× SSC (20× SSC = 0.3 M Na$_3$ citrate and 3 M NaCl, pH 7.0) with 6 M urea and 0.01 M sodium dodecyl sulfate, gently rocked in a 2× SSC solution for 5 min at room temperature, and then washed for 5 to 30 min in 2× SSC. Signal generation and detection were performed as described by the manufacturer. Filters were exposed to X-Omat film (Kodak, Rochester, N.Y.) for 1 min to 5 h. Probe hybridization was evaluated under low (2× SSC wash)- and high (0.1× SSC)-stringency conditions for each Southern blot.
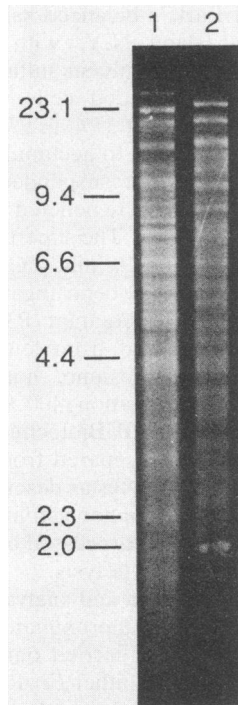
FIG. 1. Restriction patterns of total *B. gibsoni* (lane 1) and *B. microti* (lane 2) merozoite DNAs digested with *Eco*RI and fragments separated on a 0.8% agarose gel. Molecular sizes are indicated on left in kilobases.
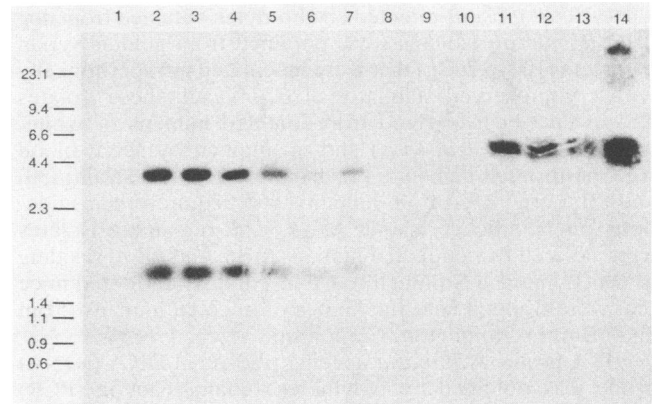

FIG. 2. Hybridization of *Hae*III-digested leukocyte and babesial merozoite DNAs with a chemiluminescent rDNA probe. Lanes: 1, canine leukocytes; 2 to 7, *B. gibsoni* isolates 4Bg (lane 2), 2Bg (lane 3), 3Bg (lane 4), 7Bg (lane 5), and 1Bg (lanes 6 and 7); 6, P1-infected erythrocytes; 7, P3 partially purified merozoites; 8, blank; 9, human leukocytes; 10, hamster leukocytes; 11 to 14 *B. microti* isolates 1Bm 3Bm 5Bm, and 6Bm, respectively. Molecular sizes are indicated in kilobases on the left.

RESULTS

Restriction endonuclease digestion of genomic DNA. Multiple discrete restriction fragments were seen on 0.8% agarose gels of babesial merozoite DNA digested with restriction enzymes and stained with ethidium bromide. These bands were not observed in leukocyte DNA from uninfected humans, hamsters, or dogs. RFLPs were discernible when merozoite DNAs from different babesial isolates were digested with *Eco*RI, *Hin*dIII, or *Sau*3A and the fragments were separated on 0.8% agarose gels. Figure 1 shows the typical restriction patterns seen with *Eco*RI digests of *B. gibsoni* (lane 1) and *B. microti* (lane 2) DNAs. Although DNAs from all of the *B. gibsoni* isolates appeared to produce similar restriction patterns, which differed from the patterns produced with DNAs from the *B. microti* isolates, restriction digests were difficult to interpret visually because of the large number of fragments present.

Detection of *B. microti* and *B. gibsoni* RFLP with rDNA probe. Since restriction digests of the different DNAs were not sufficiently distinct to serve as a reliable method for species discrimination, a *Babesia*-specific rDNA probe was evaluated by hybridization to Southern blots of restriction enzyme-digested babesial DNAs. Despite having been generated from *B. microti*, the rDNA sequence probe hybridized to DNAs from all of the babesial isolates and made possible species differentiation of the isolates on the basis of the resultant RFLPs. Unless otherwise indicated, there were no differences between the hybridization patterns seen when the same blot was subjected to low- or high-stringency wash conditions after hybridization. Figure 2 shows the typical hybridization patterns seen with *Hae*III digestion of babesial and control DNAs. The chemiluminescent rDNA probe

hybridized to restriction fragments of approximately 4.0 and 1.8 kb in DNAs from the *B. gibsoni* isolates (lanes 2 to 7) and to fragments of >23.1 kb (not apparent in all of the lanes in Fig. 2 but seen in all of the isolates with longer exposure) and 5.6 kb in the *B. microti* DNAs (lanes 11 to 14). After a longer exposure time, additional faint hybridization bands were identified at 12.4 kb in DNAs from isolates 2Bg, 3Bg, and 4Bg; at 8.0 kb in DNAs from isolates 2Bg, 3Bg, 4Bg, and 7Bg; and at 7.0 kb in DNAs from isolates 1Bg and 7Bg (data not shown). The probe hybridized to a 1.0-kb fragment in DNA preparations of dog, human, and hamster leukocytes and to two *B. gibsoni* isolates (lanes 4 and 6), probably owing to short regions of probe homology to 16S-like rRNA sequences within the leukocyte DNA.

Figure 3 shows the RFLPs seen with probe hybridization to *Hin*dIII digests of babesial and control DNAs. The most intense hybridization was to sequences in 3.1- and 2.6-kb fragments of all of the *B. gibsoni* DNAs (lanes 2 to 6) and a 1.4-kb fragment in the *B. microti* DNAs. Less intense hybridization bands were also detected in a 15.7-kb fragment in four of the five *B. gibsoni* DNAs (lanes 2 to 5). The rDNA sequence probe hybridized to a fragment of approximately 18.9 kb in DNA digests of canine leukocytes (lane 1), all of the *B. gibsoni* isolates (lanes 2 to 6), and human leukocytes (lane 8). Probe hybridization to two fragments, approximately 20.1 and 10 kb, was also detected in *Hin*dIII-digests of DNAs from hamster leukocytes (lane 9) and two of the *B. microti* isolates propagated in hamsters (lanes 12 and 13). Other faint bands, not shown in Fig. 3, were due to hybridization to a 7.7-kb fragment in DNAs from all of the *B. gibsoni* isolates (seen only after the 2× SSC washing) and a 0.7-kb fragment in all of the *B. microti* DNAs.

Results obtained with hybridization of the probe to Southern blots of *Eco*RI-digested DNA preparations are shown in Fig. 4. In *Eco*RI digests of *B. gibsoni* DNAs, rDNA sequences were detected by the probe in 8.3-kb (lanes 2 to 6) and 3.3-kb (seen with long exposure; data not shown) fragments in all of the isolates and in a 4.1-kb fragment in all of the isolates but 7Bg (lane 5). The homologous sequence was restricted to a single fragment of approximately 4.4 kb in
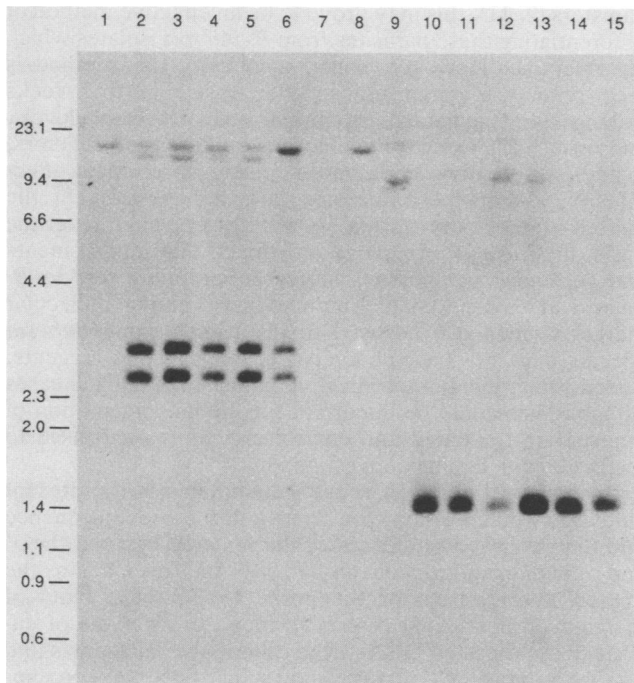
FIG. 3. Hybridization of *Hin*dIII-digested leukocyte and babe-
sial merozoite DNAs with a chemiluminescent rDNA probe. Lanes:
1, canine leukocytes; 2 to 6, *B. gibsoni* isolates 4Bg, 2Bg, 3Bg, 7Bg,
and 1Bg, respectively; 7, blank; 8, human leukocytes; 9, hamster
leukocytes; 10 to 15, *B. microti* isolates 1Bm, 3Bm, 4Bm, 5Bm,
6Bm, and 2Bm, respectively. Molecular sizes are indicated in
kilobases on the left.

*Eco*RI digests of the four human- and two rodent-derived *B.
microti* isolates tested (lanes 11 to 14; 1Bm and 4Bm isolate
data not shown). The probe sequence was detected in
fragments of 20 (data not shown) and 6.3 kb in human DNA
(lane 8) and single fragments of 7.2 and 14 kb in DNA
preparations of leukocytes from a dog (lane 1) and a hamster
(lane 9), respectively. Hybridization to sequences in a 7.2-kb
fragment in DNAs from some of the *B. gibsoni* isolates
(lanes 4 and 5) and a 14-kb fragment in some *B. microti* DNA
preparations (data not shown) indicated the presence of host
leukocyte contamination.

Similarly, hybridization patterns produced with *Sau*3A-
digested DNAs from the *B. gibsoni* isolates were distinctly
different from those produced by the *B. microti* isolates (data
not shown). The rDNA probe identified sequences in 1.6-,
1.0-, and 0.9-kb fragments in *Sau*3A-digested DNAs of the
five *B. gibsoni* isolates and in a 1.0-kb fragment in DNAs of
the human (2Bm and 3Bm) and rodent (5Bm and 6Bm) *B.
microti* isolates tested. Some variation between *B. gibsoni*
isolates was demonstrated, with hybridization occurring to
additional fragments of 1.7 and 1.4 kb in *Sau*3A-digested
DNAs from isolates 1Bg and 7Bg.

DISCUSSION

Electrophoretic separation of restriction endonuclease di-
gests of merozoite DNA showed that *B. microti* isolates from
rodents and humans had similar restriction patterns and that
these patterns differed from those produced by DNA from *B.
gibsoni* isolated from dogs. These differences were more
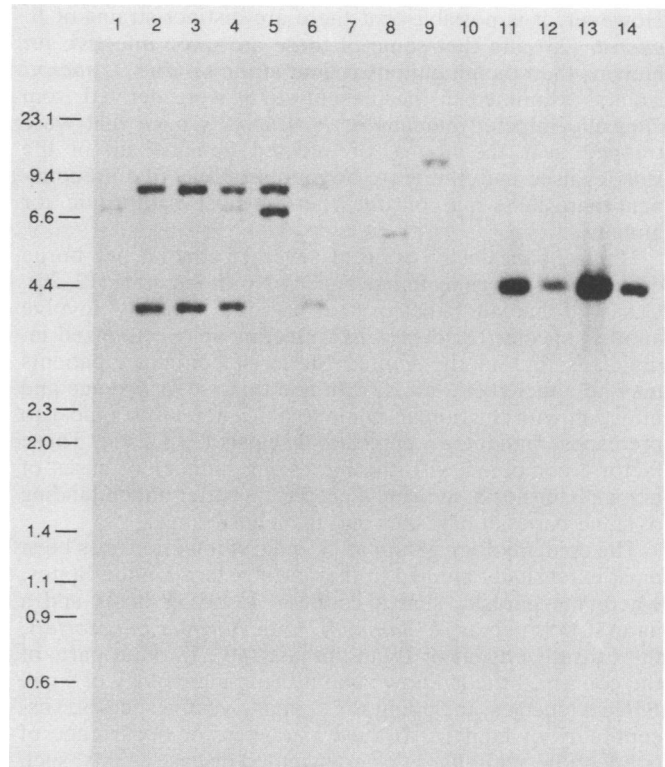clearly and consistently demonstrated by Southern blot

FIG. 4. Hybridization of *Eco*RI-digested leukocyte and babesial
merozoite DNAs with a chemiluminescent rDNA probe. Lanes: 1,
canine leukocytes; 2 to 6, *B. gibsoni* isolates 4Bg, 2Bg, 3Bg, 7Bg,
and 1Bg, respectively; 7, blank; 8, human leukocytes; 9, hamster
leukocytes; 10, blank; 11 to 14, *B. microti* isolates 3Bm, 5Bm, 6Bm,
and 2Bm, respectively. Molecular sizes are indicated in kilobases on
the left.

analysis using a probe which was produced by PCR ampli-
fication of a 16S-like rDNA sequence from *B. microti* DNA.
The probe hybridized to DNAs from isolates of both species,
and the RFLPs demonstrated distinct genotypic differences
between isolates of *B. microti* and *B. gibsoni*.

Although the predominant hybridization bands were con-
sistent among the various *B. gibsoni* DNAs, there was some
minor variation between isolates. The hybridization patterns
seen with 2Bg, 3Bg, and 4Bg were the same; however,
differences between these isolates and the 1Bg and 7Bg
isolates were apparent with *Hae*III-, *Eco*RI-, and *Sau*3A-
digested DNAs. These results are not surprising, since the
3Bg and 4Bg isolates were obtained from dogs that were
experimentally infected with blood from dog 2Bg, whereas
dogs 1Bg and 7Bg received blood from two different, natu-
rally infected dogs. Genotypic differences between isolates
and subpopulations within other *Babesia* (7, 17, 19) and
closely related *Theileria* species (1, 9, 10) have been dem-
onstrated by using genomic DNA probes. However, this is
the first report of the successful use of an rDNA sequence
probe to distinguish different isolates within the same spe-
cies and between *Babesia* species on the basis of RFLPs.

In comparison with the *B. gibsoni* isolates, the hybridiza-
tion patterns seen with DNAs from different *B. microti*
isolates were consistently similar to each other. This appar-
ent genotypic similarity between the *B. microti* isolates from
Nantucket Island and Connecticut may be biased by the
limited number of samples and the single probe evaluated.

However, it is possible that there are distinct strains of *B. microti* (25) and that some of these are more infective for humans than the ubiquitous rodent strain(s). The *B. microti* isolates examined in the present study were derived from clinically infected humans or *P. leucopus* mice that were trapped near the homes of infected humans; all of the isolates used may therefore be representative of a predominant parasite strain or one that is most pathogenic for humans.

Human babesiosis is one of several zoonotic tick-borne diseases of increasing importance in North America (12, 29). Cases of human babesiosis in Europe typically involve another species (*B. divergens*) infecting splenectomized individuals, but in the United States spleen-intact patients may be affected (12, 18, 43). In addition, advanced age and infection with the human immunodeficiency virus appear to predispose patients to clinical babesiosis (3, 33, 43). These factors, combined with the increasing number of cases of human babesiosis, indicate a need for a better understanding of small babesial parasites and their host range.

The epidemiology of human *B. microti* infections has been most extensively studied in the northeastern United States, where the parasite shares common reservoir hosts and a natural tick vector, *I. dammini*, with *Borrelia burgdorferi*, the causative agent of Lyme disease (40). In other parts of the country, little is known about the epidemiology of small *Babesia* species, including the primary vector species, susceptibility of humans to these parasites, or occurrence of coinfections with other tick-transmitted disease agents, such as *B. burgdorferi* or *Ehrlichia* spp. Rodent-derived *B. microti* has been incriminated as the cause of human babesiosis in California (5, 6, 36); however, these cases could also have resulted from infection with another small babesial parasite, such as *B. gibsoni*. Multiple cases of canine *B. gibsoni* infections have been reported in California (11), and an infected dog was identified in Connecticut (2). Distribution of this parasite is undoubtedly wider than these reports indicate, because infected dogs probably remain asymptomatic carriers of *B. gibsoni* for many years and they are likely to accompany their owners when they travel or move to different parts of the country. In addition to the natural foci of *B. gibsoni* infection which have been reported, it is estimated that hundreds of dogs a year are imported to the United States from areas in Asia where *B. gibsoni* is endemic, and many of these are potential parasite carriers (14).

Both human babesiosis and canine babesiosis are frequently misdiagnosed, particularly in areas where the causative organisms are not endemic, when autoanalyzers are used in hematology laboratories for blood cell differential counts or when medical personnel are not familiar with these parasites (6, 11). *B. microti* and *B. gibsoni* are morphologically indistinguishable on stained thin blood smears; both appear as small pleomorphic, intraerythrocytic protozoa that are usually round, oval, or pear shaped, with occasional characteristic tetrad or Maltese cross forms. Serologic results of indirect fluorescent-antibody tests, which are commonly used for diagnosis of babesial infections, can also be confusing because there is considerable antigenic cross-reactivity between *B. microti* and *B. gibsoni*. For example, some *Babesia*-infected humans and dogs with reciprocal titers of 2,560 to 40,960 to *B. microti* and *B. gibsoni* antigens, respectively, showed reciprocal titers of 320 to 2,560 to the heterologous antigen (42). The other method used to determine whether human babesiosis is due to *B. microti* is to inoculate blood from infected patients into hamsters (4). Since the *B. gibsoni* isolates tested have not grown in

hamsters (2, 11), this may prove to be an effective method of differentiating these parasites from *B. microti* isolates which are infective. However, xenodiagnosis by this method is inconvenient, requires sufficient time (usually 1 to 2 weeks or longer) for the parasites to propagate to detectable levels, and may be inconclusive because a variety of factors, including isolate variation and inoculum dose, could affect infectivity. For these reasons, human babesiosis in the United States is most often attributed to *B. microti* on the basis of parasite morphology on stained thin blood smears and reactivity by indirect fluorescent-antibody test to *B. microti* antigens. Reliable immunological and/or molecular markers which can be used to distinguish small *Babesia* species have not become available as diagnostic reagents. Specific identification of infective parasites in clinical cases of babesiosis could be important for the implementation of appropriate treatment and control procedures and used to facilitate epidemiologic investigations.

Analysis of the rDNA sequence amplified in the present study showed 75% homology between the probe sequence and the published sequences of the first 550 bp from the 5′ end of human and rodent 16S-like rRNAs. This explains the limited hybridization of the probe to Southern blots of mammalian host cell DNAs. Previous examinations of endonuclease-digested DNAs from other babesial isolates and *Theileria* parasites (10) have shown that host cell contamination can obscure the distinct banding pattern which is otherwise apparent on ethidium bromide-stained gels of electrophoretically separated restriction fragments. Host cell DNA contamination in the preparations used in the present study was apparently minimal, judging from the clarity of the restriction patterns and the limited hybridization of the probe to DNAs of the different babesial isolates. This host cell contamination did not impede our ability to differentiate babesial isolates on the basis of RFLPs. However, in the development of *Babesia* species-specific probes for future studies, sequences which lack homology to mammalian cell rRNA can be selected. Identification of *B. microti* and *B. gibsoni* sequences will be useful for generation of more specific oligonucleotide primers that can be utilized with PCR for detection of parasites in the blood of infected humans and dogs or in the tissues of infected ticks.

ACKNOWLEDGMENTS

REFERENCES

1. **Allsop, B., M. Carrington, H. Baylis, S. Sohal, T. Dolan, K. Iams.** 1989. Improved characterization of *Theileria parva* isolates using the polymerase chain reaction and oligonucleotide probes. Mol. Biochem. Parasitol. **35:**561–564.
2. **Anderson, J. F., L. A. Magnarelli, C. S. Donner, A. Spielman, and J. Piesman.** 1979. Canine *Babesia* new to North America. Science **204:**1431–1432.
3. **Benezra, D., A. E. Brown, B. Polsky, J. W. M. Gold, and D. Armstrong.** 1987. Babesiosis and infection with human immunodeficiency virus (HIV). Ann. Intern. Med. **107:**944.
4. **Brandt, T. F., G. R. Healy, and M. Welch.** 1977. Human babesiosis: the isolation of *Babesia microti* in golden hamsters.

J. Parasitol. **63:**934–937.

5. **Bredt, A. B., W. M. Weinstein, and S. Cohen.** 1981. Treatment of babesiosis in asplenic patients. J. Am. Med. Assoc. **245:**1938–1939.

6. **Bruckner, D. A., L. S. Garcia, R. Y. Shimizu, E. J. C. Goldstein, P. M. Murray, and G. S. Lazar.** 1984. Babesiosis: problems in diagnosis using auto-analyzers. Am. J. Clin. Pathol. **83:**520–521.

7. **Carson, C. A., P. Timms, A. F. Cowman, and N. P. Stewart.** 1990. *Babesia bovis*: evidence for selection of subpopulations during attenuation. Exp. Parasitol. **70:**404–410.

8. **Chisolm, E. S., T. K. Ruebush II, A. J. Sulzer, and G. R. Healy.** 1978. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. Am. J. Trop. Med. Hyg. **27:**14–19.

9. **Conrad, P. A., C. L. Baldwin, W. C. Brown, B. Sohanpal, T. T. Dolan, B. M. Goddeeris, J. C. DeMartini, and O. K. Ole-MoiYoi.** 1989. Infection of bovine T cell clones with genotypically distinct *Theileria parva* parasites and analysis of their cell surface phenotype. Parasitology **99:**205–213.

10. **Conrad, P. A., K. Iams, W. C. Brown, B. Sohanpal, and O. K. Ole-MoiYoi.** 1987. DNA probes detect genomic diversity in *Theileria parva* stocks. Mol. Biochem. Parasitol. **25:**213–226.

11. **Conrad, P. A., J. Thomford, I. Yamane, J. Whiting, L. Bosma, T. Uno, H. J. Holshuh, and S. Shelly.** 1991. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. J. Am. Vet. Med. Assoc. **199:**601–605.

12. **Dammin, G. J., A. Spielman, J. L. Benach, and J. Piesman.** 1981. The rising incidence of clinical *Babesia microti* infection. Hum. Pathol. **12:**398–400.

13. **Etkind, P., J. Piesman, T. K. Ruebush II, A. Spielman, and D. D. Juranek.** 1980. Methods for detecting *Babesia microti* infection in wild rodents. J. Parasitol. **66:**107–110.

14. **Farwell, G. E., E. K. LeGrand, and C. C. Cobb.** 1982. Clinical observations on *Babesia gibsoni* and *B. canis* infections in dogs. J. Am. Vet. Med. Assoc. **180:**507–511.

15. **Filstein, M. R., J. L. Benach, D. J. White, B. A. Brody, W. D. Goldman, C. W. Bakal, and R. S. Schwartz.** 1980. Serosurvey for human babesiosis in New York. J. Infect. Dis. **141:**518–521.

16. **Gadbaw, J. J., J. F. Anderson, M. L. Cartter, and J. L. Hadler.** 1989. Babesiosis—Connecticut. Morbid. Mortal. Weekly Rep. **38:**649–650.

17. **Gill, A. C., A. F. Cowman, N. P. Stewart, D. J. Kemp, and P. Timms.** 1987. *Babesia bovis*: molecular and biological characteristics of cloned parasite lines. Exp. Parasitol. **63:**180–188.

18. **Gombert, M. E., E. J. C. Goldstein, J. L. Benach, M. J. Tenenbaum, E. Grunwaldt, M. H. Kaplan, and L. K. Eveland.** 1982. Human babesiosis; clinical and therapeutic considerations. J. Am. Med. Assoc. **248:**3005–3007.

19. **Jasmer, D. P., D. W. Reduker, W. L. Goff, D. Stiller, and T. C. McGuire.** 1990. DNA probes distinguish geographical isolates and identify a novel DNA molecule of *Babesia bovis*. J. Parasitol. **76:**834–841.

20. **Kawasaki, E. S.** 1990. Sample preparation from blood, cells and other fluids, p. 146–152. *In* M. A. Innis, D. H. Gelfand, T. J. Sninsky, and T. T. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., New York.

21. **Krause, P. J., S. R. Telford III, R. Raymond, A. B. Hurta, I. Kwasnik, S. Luger, J. Niederman, M. Gerber, and A. Spielman.** 1991. Geographical and temporal distribution of babesial infection in Connecticut. J. Clin. Microbiol. **29:**1–4.

22. **Levine, N. D.** 1985. Apicomplexa: the piroplasms, p. 291–328. *In* N. D. Levine (ed.), Veterinary protozoology. Iowa State University Press, Ames.

23. **Medlin, L., H. J. Elwood, S. Stickel, and M. L. Sogin.** 1988. The characterization of enzymatically amplified eukaryotic 16S-like coding regions. Gene **71:**491–499.

24. **Moore, J. A., and R. E. Kuntz.** 1981. *Babesia microti* infections in nonhuman primates. J. Parasitol. **67:**454–456.

25. **Moss, D. M., G. R. Healy, J. W. Dickerson, and H. M. Mathews.** 1986. Isoenzyme analysis of *Babesia microti* infections in humans. J. Protozool. **33:**213–215.

26. **Murphy, T. M.** 1986. Effects of rapid passage in the gerbil (*Meriones unguiculatus*) on the course of infection of the bovine piroplasm *Babesia divergens* in splenectomized calves. Res. Vet. Sci. **40:**285–287.

27. **Persing, D. H., S. R. Telford III, D. Mathiesen, A. Spielman, W. F. Marshall, J. Thomford, and P. A. Conrad.** Submitted for publication.

28. **Phillips, R. S.** 1984. *Babesia divergens* in splenectomized rats. Res. Vet. Sci. **36:**251–255.

29. **Piesman, J.** 1987. Emerging tick-borne diseases in temperate climates. Parasitol. Today **3:**197–199.

30. **Piesman, J., S. J. Karakashian, S. Lewengrub, M. A. Rudzinska, and A. Spielman.** 1986. Development of *Babesia microti* sporozoites in adult *Ixodes dammini*. Int. J. Parasitol. **16:**381–385.

31. **Popovsky, M. A., L. E. Lindberg, A. L. Syrek, and P. L. Page.** 1988. Prevalence of *Babesia* antibody in a selected blood donor population. Transfusion (Philadelphia) **28:**59–61.

32. **Raucher, H. S., H. Jaffin, and J. L. Glass.** 1984. Babesiosis in pregnancy. Obstet. Gynecol. **63:**75–95.

33. **Ruebush, T. K., D. D. Juranek, A. Spielman, J. Piesman, and G. R. Healy.** 1981. Epidemiology of human babesiosis on Nantucket Island. Am. J. Trop. Med. Hyg. **30:**937–941.

34. **Ruebush, T. K., II, J. Piesman, W. E. Collins, A. Spielman, and M. C. Warren.** 1981. Tick transmission of *Babesia microti* to rhesus monkeys (*Macaca mulatta*). Am. J. Trop. Med. Hyg. **30:**555–559.

35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

36. **Scholtens, R. G., E. H. Braff, G. R. Healy, and N. Gleason.** 1968. A case of babesiosis in man in the United States. Am. J. Trop. Med. Hyg. **17:**810–813.

37. **Sebeck, Z., B. Rosicky, and W. Sixl.** 1977. The occurrence of babesiosis affecting small terrestrial mammals and the importance of this zoonosis in Europe. Folia Parasitol. **24:**221–228.

38. **Smith, D. R., and R. R. Clevenger.** 1985. *Babesia microti* in an Indiana woman. Indiana Med. March:212–214.

39. **Spielman, A.** 1976. Human babesiosis on Nantucket Island: transmission by nymphal Ixodes ticks. Am. J. Trop. Med. Hyg. **25:**784–787.

40. **Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman.** 1985. Ecology of *Ixodes dammini*-borne human babesisosis and Lyme disease. Annu. Rev. Entomol. **30:**439–460.

41. **Steketee, R. W., M. R. Eckman, E. C. Burgess, J. N. Kuritsky, J. Dickerson, W. L. Schell, M. S. Godsey, and J. P. Davis.** 1985. Babesiosis in Wisconsin. J. Am. Med. Assoc. **253:**2675–2678.

42. **Thomford, J., I. Yamane, and P. A. Conrad.** Unpublished data.

43. **Villar, B. F., D. J. White, and J. L. Benach.** 1991. Human babesiosis, p. 129–143. *In* T. Sun (ed.), Progress in clinical parasitology. Field & Wood Medical Publishers, Inc., New York.

44. **Wiger, R.** 1978. Fatal experimental *Babesia microti* infections in the Norwegian lemming, *Lemmus lemmus* (L.). Folia Parasitol. **25:**103–108.