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Cysticercosis results from ingestion of the eggs of the tapeworm Taenia solium. Reduction of the incidence of human and swine cysticercosis requires identification and treatment of individuals who carry the adult tapeworm. T. solium and Taenia saginata eggs cannot be differentiated on the basis of morphology; thus, in order to improve existing methods for the diagnosis of taeniasis, we have developed highly sensitive, species-specific DNA probes which differentiate T. solium and T. saginata. Recombinant clones containing repetitive DNA sequences which hybridize specifically with genomic DNAs from either species were isolated and characterized. T. solium-specific DNA sequences contained complete and truncated forms of a tandemly repeated 158-bp DNA sequence. An unrelated T. saginata DNA sequence was also characterized and shown to encode a portion of the mitochondrial cytochrome c oxidase I gene. T. solium- and T. saginata-specific DNA probes did not hybridize in dot blot assays either with genomic DNA from the platyhelminths Taenia hydatigena, Taenia pisiformis, Taenia taeniaeformis, Echinococcus granulosus, and Schistosoma mansoni or with genomic DNA from other eukaryotes, including Saccharomyces cerevisiae, Candida albicans, Cryptosporidium parvum, Entamoeba histolytica, Trypanosoma gambiensae, Trypanosoma brucei, and Giardia lamblia, Caenorhabditis elegans, and human DNA. By using these T. solium and T. saginata DNA probes, a rapid, highly sensitive and specific dot blot assay for the detection of T. solium eggs was developed.

Taenia solium and Taenia saginata are responsible for the two major forms of taeniasis in humans (20). T. solium is the more severe human pathogen, causing cysticercosis, a disease of particular importance in many developing countries (12, 19). Recently, the incidence of cysticercosis has also risen in some developed countries because of migration and tourism (6, 7). T. solium causes taeniasis in humans after the ingestion of infected pork. The clinical symptoms are not serious, and frequently Taenia carriers are asymptomatic (11, 16, 20). On the other hand, cysticercosis results from ingestion of T. solium eggs which are commonly shed by a household member harboring an adult tapeworm (25–27). Neurocysticercosis is the most common and serious form of cysticercosis in humans (12, 16). Control of this disease is dependent on reduction of the contamination of water and food by T. solium eggs. Therefore, a promising strategy to decrease the incidence of cysticercosis is to identify and treat the carriers of the adult stage of this tapeworm.

Diagnosis of taeniasis is routinely performed by coproscopical examination (11), which has a low sensitivity of detection (10). Because T. solium and T. saginata eggs have identical morphologies (11, 16), this method also lacks specificity. The beef tapeworm, T. saginata, does not cause human cysticercosis; thus, its differentiation from T. solium has epidemiological as well as clinical importance. While a high degree of sensitivity has been achieved with an enzyme-linked immunosorbent assay (ELISA) for coproantigen detection of human Taenia carriers (1), this assay cannot distinguish T. solium from T. saginata (1). DNA probes offer the possibility of a sensitive, rapid, and noninvasive diagnostic test which could distinguish between T. solium carriers and T. saginata carriers. Diagnosis using repetitive DNA probes, which results in improved sensitivity, has been successful for Trypanosoma cruzi (17), Leishmania (30), Plasmodium falciparum (4), Entamoeba histolytica, Trypanosoma gambiensae, Trypanosoma brucei, and Giardia lamblia, Caenorhabditis elegans, and human DNA. In this paper, we describe two such repetitive DNA sequences isolated from T. solium and T. saginata and demonstrate the utility of these probes for the detection of T. solium eggs.

MATERIALS AND METHODS

Parasites. T. solium cysticerci, which were obtained from the carcass of a naturally infected pig, were immediately frozen and stored at −20°C. An adult T. saginata worm from a patient was obtained and stored in 70% ethanol. Several gravid proglottids from two adult T. solium worms obtained in China in 1988 and from two worms collected in Mexico in 1992 were kept in 70% ethanol.

Isolation of DNA. Parasite DNA was prepared by a modification of the protocol described by Sambrook et al. (22). Briefly, frozen parasites were placed in a mortar that was precooled and partially filled with liquid nitrogen and were ground to a fine powder with a chilled pestle. The powder was suspended in DNA extraction buffer (50 mM Tris-HCl [pH 7.5], 50 mM EDTA [pH 8.0], 0.5% [wt/vol] sodium dodecyl sulfate [SDS], proteinase K [200 µg/ml]), and the suspension was incubated at 55°C for 60 min. DNA was extracted twice with an equal volume of phenol-chloroform-isooamyl alcohol (25:24:1). Nucleic acids were precipitated with 95% ethanol and highly concentrated salt at −20°C for 1 h before being harvested by centrifugation at 12,000 × g. The pellet was suspended in TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), RNase A was added to a 20-µg/ml final concentration, and the mixture was incubated at 37°C for 30 min. The reaction mixture was then extracted and subjected to ethanol precipitation as described above.
Construction and screening of genomic DNA libraries. Genomic DNA from T. solium or T. saginata was partially restricted with Sau3AI to yield fragments ranging from 300 bp to approximately 5 kb. The fragments were cloned into the BamHI site of pBluescript KS (+/−) (Stratagene, La Jolla, Calif.) and were amplified in Escherichia coli host XL-1 T. solium and T. saginata plasmid libraries of 2.8 × 10⁶ and 3.9 × 10⁶ recombinant clones, respectively, each contained an insert of 0.3 to >7 kb. Approximately 3,000 colonies from each library were screened for repetitive sequences by colony hybridization (18) with total genomic T. solium or T. saginata DNA which had been ³²P labelled by random primer extension (22). Those recombinant clones which hybridized strongly with the probe were chosen for further characterization.

Analysis of recombinant clones. ³²P-labelled plasmid DNA probes prepared from recombinant clones by random hexamer primer extension (22) were used for Southern analysis of Taenia genomic DNAs. Genomic DNAs prepared from T. solium, T. saginata, Taenia hydatigena, Taenia pisiformis, and Taenia taeniaeformis were digested with several endonucleases, separated in agarose gels, and immobilized on either nitrocellulose or Nytran filters (Schleicher & Schuell, Keene, N.H.) (29) as recommended by the manufacturer. Hybridizations were performed at 42°C overnight in 50% formamide, 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt’s solution (5× Denhardt’s solution is 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), and denatured salmon sperm DNA (10 μg/ml). The filters were washed three times with 2× SSC-0.1% SDS at 65°C prior to a single washing with 0.1× SSC-0.1% SDS at 65°C. Hybridization of labelled probes was visualized by autoradiography. Clones showing species-specific hybridization were selected for further analysis and DNA sequencing. Recombinant clones were sequenced with chain terminators by an automated sequencing protocol (Applied Biosystems) and extension (22). Those recombinant clones which hybridized strongly with the species-specific probes as described above. The probes were also evaluated for specificity and sensitivity in dot blot assays using serial dilutions of genomic DNAs. Genomic DNAs from T. solium, T. saginata, T. hydatigena, T. pisiformis, and T. taeniaeformis were digested with several endonucleases, separated in agarose gels, and immobilized on either nitrocellulose or Nytran filters (Schleicher & Schuell, Keene, N.H.) (29) as recommended by the manufacturer. Hybridizations were performed at 42°C overnight in 50% formamide, 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt’s solution (5× Denhardt’s solution is 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), and denatured salmon sperm DNA (10 μg/ml). The filters were washed three times with 2× SSC-0.1% SDS at 65°C prior to a single washing with 0.1× SSC-0.1% SDS at 65°C. Hybridization of labelled probes was visualized by autoradiography. Clones showing species-specific hybridization were selected for further analysis and DNA sequencing. Recombinant clones were sequenced with chain terminators by an automated sequencing protocol (Applied Biosystems) (24). Sequence comparisons were done by the Pearson and Lipman method (21) with the GenBank database.

Dot blot analysis. Preheated (95°C) genomic DNAs from T. hydatigena, T. pisiformis, T. taeniaeformis, Echinococcus granulosus, Schistosoma mansoni, Cryptosporidium parvum, and Homo sapiens were spotted onto Nytran filters, baked, and hybridized with the species-specific probes as described above. The probes were also evaluated for specificity and sensitivity in dot blot assays using serial dilutions of PCR-amplified T. solium eggs isolated from gravid segments as described previously (13). The conditions used for denaturation of purified eggs were determined empirically and consisted of adding 4 volumes of 0.2 N NaOH and boiling for 10 min. The eggs were then chilled in ice, neutralized, immobilized on a Zeta-probe membrane (Bio-Rad, Richmond, Calif.), and hybridized with the radioactive probes as described above.

RESULTS

Isolation and characterization of recombinant DNA probes. In order to clone Taenia repetitive DNA sequences, T. solium and T. saginata genomic libraries were prepared as described in Materials and Methods. The genomic DNAs used to make these libraries were labelled by random primer extension, and the probes thus generated were used to screen the corresponding libraries for high-abundance sequences. Forty recombinant clones were isolated from each genomic library and used as probes in Southern analysis to assay for species-specific hybridization.

T. solium recombinant clones. Approximately half of the clones hybridized equally well with genomic DNAs from T. solium and T. saginata (data not shown), and these were not investigated further. The remaining clones from the T. solium library hybridized predominantly with T. solium DNA. As determined by Southern blotting, many of these clones hybridized to the same small T. solium DNA band generated after HaeIII digestion (shown for pTsol-9 in Fig. 1), suggesting that these clones contain a common repeated DNA sequence. One of these clones, pTsol-9, was chosen for further analysis. To confirm that many of the T. solium-specific recombinant clones contained the same repetitive sequence, we used the pTsol-9 insert as a hybridization probe with Southern blot filters containing all the recombinant clones. As expected, many inserts from recombinant clones (11 of 31) hybridized with the probe (data not shown), suggesting that the sequence contained within the approximately 400-bp pTsol-9 insert was represented frequently in the T. solium genome. The specificity of the pTsol-9 probe was assayed by Southern blot hybridization to DNA from other taenid species (Fig. 1). In addition to the prominent hybridization to T. solium DNA, faint hybridization was detected in restriction digests containing T. saginata DNA but not in T. hydatigena, T. pisiformis, or T. taeniaeformis digests. The presence of a single predominant hybridizing band in the HaeIII-digested T. solium genomic DNA lane suggested that this repeat uniformly contained HaeIII restriction sites. Restriction mapping of pTsol-9 confirmed the presence of a repeat structure (Fig. 2A). The repeat spans 158 bp and is tandemly organized, with four HaeIII sites and a single BamHI site per repeat unit. The nucleotide sequence of a pTsol-9 repeat is shown in Fig. 2B.

T. saginata recombinant clone. One clone, pTsag-16, with an insert size of 967 bp, contained a repetitive sequence that was specific for T. saginata as determined by Southern blot analysis; there was very little cross-hybridization to T. solium DNA (Fig. 3). The pTsag-16 restriction map and nucleotide sequence are shown in Fig. 4A and B, respectively. Surprisingly, when the sequence was used to search the GenBank database by the Pearson and Lipman method (21), extensive homology between a 629-bp Sau3AI fragment and the mitochondrial gene cytochrome c oxidase I (CO1), including up to 70% identity with the platyhelminth Fasciola hepatica (14), was found. This region of homology is flanked by a 347-bp sequence which shows no homology to the CO1 gene, which suggests that the pTsag-16 clone contains two separate inserts, a mitochondrial CO1 gene fragment (bp 347 to 967) and a repeated DNA fragment (bp 1 to 346), ligated at their Sau3AI ends during the generation of the T. saginata genomic library. This hypothesis was confirmed by the absence in Southern blots of a predicted 550-bp HindIII fragment spanning this Sau3AI site (Fig. 3). These two unrelated inserts were isolated to be used as probes for Southern analysis (Fig. 5). Despite the strong evolutionary conservation of the CO1 mitochondrial gene, when the T. saginata CO1 gene fragment and the repetitive DNA fragment were hybridized separately with T. saginata and T. solium genomic DNA, both fragments showed a high degree of specificity for T. saginata DNA, with minimal cross-hybridization to T. solium DNA (Fig. 5).

Specificity of pTsol-9 and pTsag-16 in dot blots. Results of
dot blot analysis are shown in Fig. 6. pTsol-9 hybridizing sequences are much more abundant in the *T. solium* genome than in that of *T. saginata* (Fig. 6, lefthand panel). Conversely, the pTsag-16 insert was more abundant in the *T. saginata* genome than in that of *T. solium* (Fig. 6, righthand panel). Dot blot analysis was also performed with DNAs from several eukaryotic pathogens which are coendemic with *Taenia* species in some regions and thus might confound diagnostic assays. pTsol-9 did not hybridize to purified genomic DNA from *E. histolytica*, *E. granulosus* (Fig. 6, lefthand panel), *S. mansoni*, *C. elegans*, *T. brucei*, *T. gambiense*, *G. lamblia*, *S. cerevisiae*, *C. albicans*, *C. parvum*, or *Homo sapiens* (data not shown). The pTsag-16 probe did show some faint hybridization with *E. granulosus* (Fig. 6, righthand panel).

**Sensitivity and specificity of the recombinant probes on purified *T. solium* eggs.** The sensitivity of the recombinant probes for diagnostic purposes was analyzed in dot blots of serial dilutions of *T. solium* eggs (Fig. 7). The assay demonstrated remarkable sensitivity, with as little as one *T. solium* egg detected by the pTsol-9 probe. The degree of specificity was also high, since no cross-hybridization with pTsag-16 occurred. Hybridization was less sensitive when eggs obtained in China that had been stored for several years were used, and no signal was detected when eggs obtained recently in Mexico from a proglottid that was not properly preserved were used.

**DISCUSSION**

The use of DNA probes for the diagnosis of infectious agents has been successful for *T. cruzi* (17), *Leishmania* (30), *P. falciparum* (4), *E. histolytica* (8, 15, 23), *O. volvulus* (9, 28), and other infections. Most of these probes are repetitive sequences, resulting in highly sensitive hybridization assays. The screening and characterization of plasmid genomic DNA libraries prepared from both *T. solium* and *T. saginata* for species-specific repetitive DNA sequences resulted in the identification of a repetitive sequence within *T. solium* (pTsol-9) which can be used to distinguish between these two organisms. This sequence was present in approximately 30% of the *T. solium* species-specific clones. Structural analysis of some of these clones indicates that this repetitive sequence is tandemly arranged and contains *HaeIII* fragments of 76, 61, and 8 bp which are superimposed as a single prominent band in Southern blots.

An apparently unrelated *T. saginata*-specific DNA probe, pTsag-16, contains at least two unrelated sequences: a repetitive *T. saginata* sequence and a fragment of the *T. saginata* mitochondrial CO1 gene. Both of these sequences are species specific in their hybridization. Comparison of the nucleotide sequence and the predicted amino acid sequence of the *T. saginata* CO1 gene to sequences extracted from GenBank indicated that the *T. saginata* CO1 gene shares 70% identity in both nucleotide and amino acid sequences with the trematode *F. hepatica* CO1 gene (14). Although sequences from a region of the CO1 gene have been reported by Bowles et al. for the cestode genus *Echinococcus* (5), which is more closely related to *T. saginata* than is *F. hepatica*, no direct comparisons with our reported *T. saginata* CO1 gene fragment could be made since our sequence derives from a different region of the CO1 gene. With such extensive homology of the *T. saginata* CO1 gene to that of *F. hepatica*, which belongs to a different class of platyhelminths, we would expect even greater homology to another cestode such as *T. solium*. This expected homology may explain the faint signals that were detected in *T. solium* DNA by pTsag-16, although when probed separately, both fragments (bp 1 to 346 and 347 to 997) yielded minimal cross-reactivity to *T. solium* DNA. As seen with the *HaeIII* digests, the approximately 16-kb CO1 signal (Fig. 5) represents a significant fraction of the total pTsag-16 signal seen in Fig. 3. This 16-kb CO1 band is likely to represent mitochondrial...
While the CO1 gene is probably a single-copy mitochondrial gene, its intensity on a Southern blot might be expected to approach that seen with the 346-bp repetitive sequence, depending on the average number of mitochondria per cell.

Slight cross-hybridization with the pTsol-9 insert was also found in Southern blots containing T. saginata genomic DNA. This slight cross-hybridization may be nonspecific, may be the result of a low copy number of the repeat in the heterologous DNA, or may be because of the presence of a structurally similar but divergent sequence. No attempts to distinguish between these possibilities have been made. Although this cross-hybridization may preclude the use of either DNA probe alone, when used in parallel the probes provide unambiguous distinction between T. solium and T. saginata. Furthermore, both probes were entirely specific in dot blots performed with DNA from other Taenia species as well as several other eukaryotic DNAs likely to be found in stool samples.

FIG. 4. (A) Restriction map of clone pTsag-16. S, Sau3AI; E, EcoRI; H, HindIII; Ps, PstI; PvuII; X, XbaI. The open box contains sequences homologous to the mitochondrial CO1 gene (Cyt Ox I). (B) DNA sequence of the 967-bp clone, pTsag-16.

FIG. 5. Southern blot of Taenia genomic DNA probed with separate fragments of the pTsag-16 clone. Each lane contains 5 µg of genomic restricted DNA from either T. solium or T. saginata that was submitted to electrophoresis in a 1% agarose gel, immobilized on nitrocellulose filters, and hybridized overnight with [32P]dCTP-labelled pTsag-16 fragments. The CO1 probe was the 621-bp Sau3AI fragment, while the 5′ pTsag-16 probe was the 348-bp XbaI-PstI fragment. The sizes of significant size markers are indicated on the left.

FIG. 6. Dot blot analysis of genomic DNA from different taenid species. After dilution, the amounts of genomic DNA indicated below each lane were applied to Nytran filters. The left panel was probed with pTsol-9, and the right panel was probed with pTsag-16.
The dot blot analysis of *T. solium* eggs suggests that the pTsol-9 and pTsag-16 repetitive sequences when used in conjunction should provide specific and sensitive DNA probes for the detection of *T. solium* eggs. It will be necessary to determine the optimum storage conditions for samples, since the data in Fig. 7 indicate that there is some deterioration in the signal over time. The eggs used for columns 1 and 2 were the same ones that were used several years ago to standardize the DNA hybridization assay (13). When used at that time, even 1 egg could be detected, while now 50 or more are needed. Meanwhile, care should be taken in the use of recent samples and proper sample preservation in order to obtain a good signal in the assay. These probes show promise for the discrimination of stool samples containing *T. solium* from those containing *T. saginata* that neither coproscopical nor antigen detection assays are able to achieve (1, 11, 16). Further studies are needed to optimize the sensitivity and specificity of these probes when used with clinical stool specimens. Once developed, such a clinical assay will need to be evaluated by clinical trials comparing DNA hybridization with coproscopical and clinical evaluations. Such a clinical assay would optimally rely on one of the nonradioactive detection reagents without the need of special equipment. In this way, the test could also be implemented for epidemiological studies and could probably be performed in field conditions. A coproantigen ELISA in a dipstick presentation was recently used in such field conditions and proved to be quite useful (2).

As has recently been shown for *P. falciparum* diagnosis, DNA probe hybridization procedures with clinical specimens can be performed in laboratories of both developed and developing countries with similar results (3). The cost of these assays (U.S. S0.17 per assay) was approximately half that of microscopic examination. Although assays of stool samples are likely to be more difficult to develop, we would expect similar results from a clinical assay based on the *Taenia* probes reported here since DNA hybridization is more sensitive and easier to perform than a routine coproscopical examination and requires no expertise in microscopical observation. The ability to differentiate the species of *Taenia* eggs has important epidemiological implications, since *T. solium* causes human neurocysticercosis while *T. saginata* does not and the main risk factor for acquiring cysticercosis is the presence of a *T. solium* carrier within the household (25, 27). Thus, the primary aim in developing such a clinical DNA hybridization test is to apply it in neurocysticercosis control campaigns which aim to detect and treat *T. solium* tapeworm carriers. The test would also be useful in areas where the parasite is nonendemic to screen immigrants for *T. solium* tapeworm carriage in order to prevent the kind of locally acquired neurocysticercosis recently found in New York City (27).

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