

# Isolation and Characterization of Species-Specific DNA Probes from *Taenia solium* and *Taenia saginata* and Their Use in an Egg Detection Assay

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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 gambiense*, *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. sagi-*

*nata* (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* (8, 15, 23), and *Onchocerca volvulus* (9, 28) infections, among others.

In order to develop a specific diagnostic assay for *T. solium* intestinal infection, we have applied recombinant DNA techniques to isolate specific and sensitive diagnostic DNA probes. 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  $-20^{\circ}\text{C}$ . 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 México 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  $\mu\text{g}/\text{ml}$ ]), and the suspension was incubated at  $55^{\circ}\text{C}$  for 60 min. DNA was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). Nucleic acids were precipitated with 95% ethanol and highly concentrated salt at  $-20^{\circ}\text{C}$  for 1 h before being harvested by centrifugation at  $12,000 \times g$ . The pellet was suspended in TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), RNase A was added to a 20- $\mu\text{g}/\text{ml}$  final concentration, and the mixture was incubated at  $37^{\circ}\text{C}$  for 30 min. The reaction mixture was then extracted and subjected to ethanol precipitation as described above.

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**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 *Bam*HI 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  $2.8 \times 10^7$  and  $3.9 \times 10^7$  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  $^{32}$ P labelled by random primer extension (22). Those recombinant clones which hybridized strongly with the probe were chosen for further characterization.

**Analysis of recombinant clones.**  $^{32}$ P-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,  $6\times$  SSC ( $1\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate),  $5\times$  Denhardt's solution ( $5\times$  Denhardt's solution is 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), and denatured salmon sperm DNA (10  $\mu$ g/ml). The filters were washed three times with  $2\times$  SSC–0.1% SDS at 65°C prior to a single washing with  $0.1\times$  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*, *Caenorhabditis elegans*, *E. histolytica*, *Trypanosoma gambiense*, *Trypanosoma brucei*, *Giardia lamblia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Cryptosporidium parvum*, or *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 purified *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 *Hae*III 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

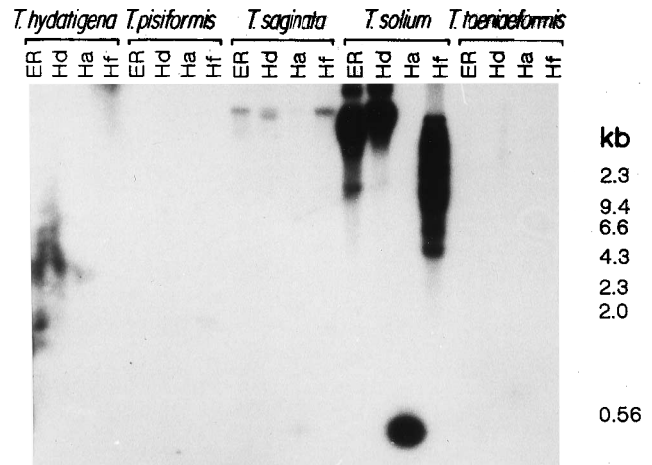


FIG. 1. Southern blot analysis of *Taenia* DNAs with the pTsol-9 recombinant clone isolated from *T. solium* genomic DNA library as a probe. Each lane contains 5  $\mu$ g of genomic restricted DNA from different taeniid species that was submitted to electrophoresis in 1.0% agarose gels, immobilized on Nytran filters, and hybridized overnight with a [ $^{32}$ P]dCTP-labelled pTsol-9 insert. ER, *Eco*RI; Hd, *Hind*III; Ha, *Hae*III; Hf, *Hinf*I.

to DNA from other taeniid 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 *Hae*III-digested *T. solium* genomic DNA lane suggested that this repeat uniformly contained *Hae*III 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 *Hae*III sites and a single *Bam*HI 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 *Sau*3AI fragment and the mitochondrial gene cytochrome *c* oxidase I (CO 1), 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 CO 1 gene, which suggests that the pTsag-16 clone contains two separate inserts, a mitochondrial CO 1 gene fragment (bp 347 to 967) and a repeated DNA fragment (bp 1 to 346), ligated at their *Sau*3AI ends during the generation of the *T. saginata* genomic library. This hypothesis was confirmed by the absence in Southern blots of a predicted 350-bp *Hinf*I fragment spanning this *Sau*3AI 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 CO 1 mitochondrial gene, when the *T. saginata* CO 1 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

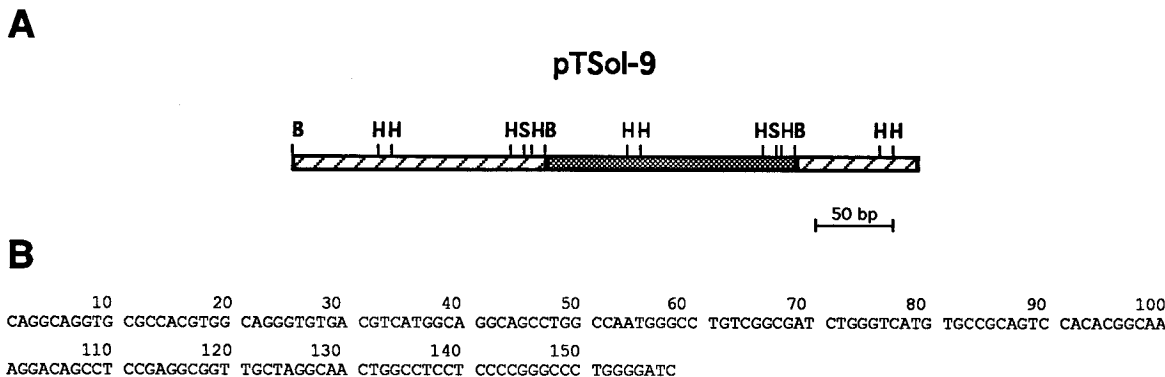


FIG. 2. (A) Restriction map of clone pTsol-9. H, *Hae*III; S, *Sma*I; B, *Bam*HI. The darkened segment highlights a single 158-bp repeat unit. (B) DNA sequence of the first 158-bp repeat of pTsol-9.

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 *Hae*III 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 CO 1 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* CO 1 gene to sequences extracted from GenBank indicated that the *T. saginata* CO 1 gene shares 70% identity in both nucleotide and amino acid sequences with the trematode *F. hepatica* CO 1 gene (14). Although sequences from a region of the CO 1 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* CO 1 gene fragment could be made since our sequence derives from a different region of the CO 1 gene. With such extensive homology of the *T. saginata* CO 1 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 *Hae*III digests, the approximately 16-kb CO 1 signal (Fig. 5) represents a significant fraction of the total pTsag-16 signal seen in Fig. 3. This 16-kb CO 1 band is likely to represent mitochondrial

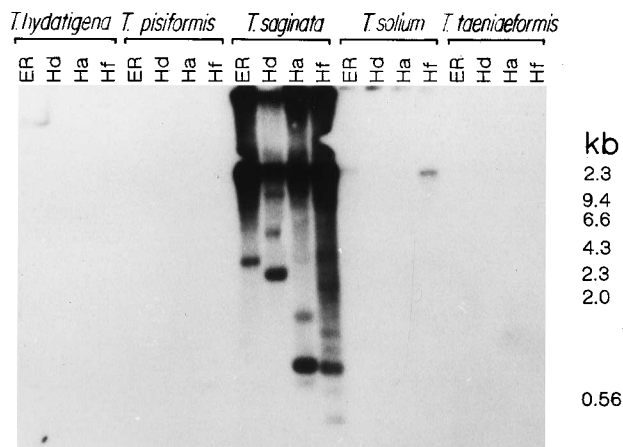
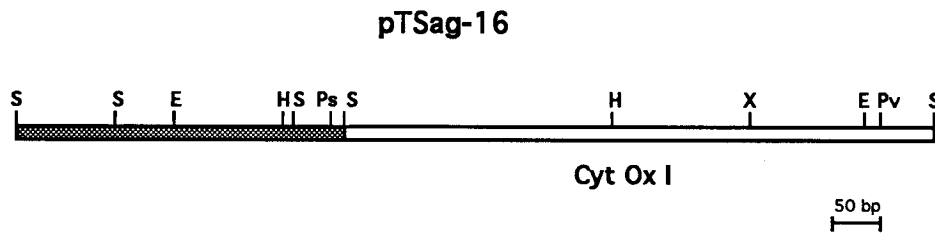


FIG. 3. Southern blot analysis of *Taenia* DNAs with the pTsag-16 recombinant clone isolated from *T. saginata* genomic DNA library as a probe. Each lane contains 5 µg of genomic restricted DNA from different taeniid species that was submitted to electrophoresis in 1.0% agarose gels, immobilized on Nytran filters, and hybridized overnight with [<sup>32</sup>P]dCTP-labelled pTsag-16. ER, *Eco*RI; Hd, *Hind*III; Ha, *Hae*III; Hf, *Hinf*I.

**A**



**B**

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10      20      30      40      50      60      70      80      90      100
CAAGCTCGCA CAGAAACACT GGGTTGTAAC CGAGAGATGC GGCTTCAGTG CTATCGTGGG TGCCATGTCC TGGTGTGTG TATGTGAATA CGGACGCTGT
110     120     130     140     150     160     170     180     190     200
CAAGATCGCC GTGTGTTTCC ATTACGTCCT GTCGATGGAA TAGCCACTTC TTCCTTTTCC ACCTCTTCCT TCTCACCCT CCTGCTATGG GAGAATTGGA
210     220     230     240     250     260     270     280     290     300
CGTTTTTGAA CATCTTTATT ACAATCATA GAGGATGTG TGGTGGCGAA ACCGAGACAG TTAAGACAAG GGTATCTCCG AGTCCGCAGA GCGATCACCA
310     320     330     340     350     360     370     380     390     400
ACACGAAATG GCATGCAGTG AGAGCGAAAT CTGCAGTGTG GTGAAGATCA TAAGCGGGTT GGTATGATTT ATACTTTGTT GGGTTTGTGG TCAGGTTTTG
410     420     430     440     450     460     470     480     490     500
TAGGTTTAAG ATTTAGTTTA TTAATTCGTG TTAATTTTTT AGAGCCTTAT TATAATGTGA TTCTTTGGA TTGTATAAT TTTTGTATA CTAATCATGG
150     520     530     540     550     560     570     580     590     600
AATAAATAATG ATTTTCTTTT TTTTAATGCC TATTTAATAA GGTGGTTTCG GCAAATATTT AATTCCTTTG GTTGGTGGGT TATCTGATTT GAATTTACCC
610     620     630     640     650     660     670     680     690     700
CGTTTAAATG CTTTAAGTGC ATGGTTATTG ATTCCCTCGA TGGCTTTTCT TTTGGTTAGT ATGTGTTTGG GTGCTGGTAT AGGGTGGACT TTTTATCCGC
710     720     730     740     750     760     770     780     790     800
CTTTGTGCTC ATCATTATTT TCAAGTAGTA ATGGTGTGGA TTTCTTGATG TTTTCGTGTC ATTTGGCGGG TCGCTCTAGA ATTTTATGTT CTATTAATTT
810     820     830     840     850     860     870     880     890     900
TATTTGTAAT TTGTATAGAA TATTTATGAC TAATATATTT TCTCGTACTT CTATAATATT GTGGGCTTAT TTATTTACGT CTATTTTATT ATTAGTTACT
910     920     930     940     950     960
CTTCCCTGAT TAGCAGCTGC TATCACTATG CTTTTATTTC ACCGTAATTT TAGTTCCTGCG TTTTTTTG
    
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FIG. 4. (A) Restriction map of clone pTSag-16. S, *Sau3AI*; E, *EcoRI*; H, *HinfI*; Ps, *PstI*; Pv, *PvuII*; X, *XbaI*. The open box contains sequences homologous to the mitochondrial CO 1 gene (*Cyt Ox I*). (B) DNA sequence of the 967-bp clone, pTSag-16.

DNA monomers. While the CO 1 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.

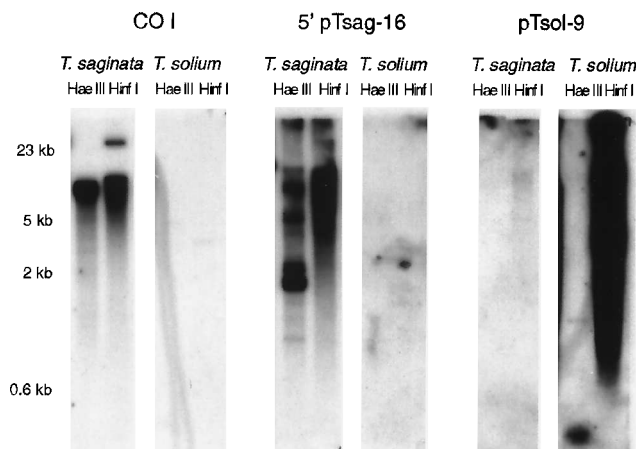


FIG. 5. Southern blot of *Taenia* genomic DNA probed with separate fragments of the pTsag-16 clone. Each lane contains 5 µg of genomic restricted DNA from either *T. solium* or *T. saginata* that was submitted to electrophoresis in a 1.0% agarose gel, immobilized on nitrocellulose filters, and hybridized overnight with [<sup>32</sup>P]dCTP-labelled pTsag-16 fragments. The CO 1 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.

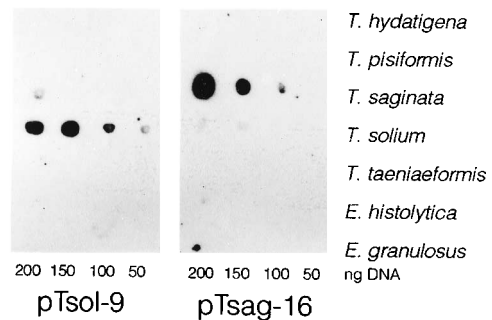


FIG. 6. Dot blot analysis of genomic DNA from different taeniid species. After dilution, the amounts of genomic DNA indicated below each lane were applied to Nytran filters. The left panel was probed with pTsol-9, and the right panel was probed with pTsag-16.

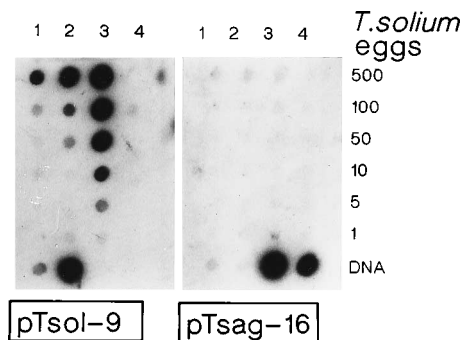


FIG. 7. Dot blot analysis of *T. solium* eggs dotted in different amounts. The row marked DNA contains 1 ng of *T. solium* DNA (columns 1), pTsol-9 (columns 2), pTsag-16 (columns 3), and *T. saginata* DNA (columns 4). Eggs obtained in China and kept in 70% ethanol since 1988 (columns 1 and 2) and eggs obtained in Mexico in 1992 (columns 3 and 4) which were also kept in 70% ethanol, were used (the specimen used to isolate eggs from sample 4 was not well preserved). The left and right panels were hybridized with  $^{32}\text{P}$ -labelled pTsol-9 and Tsag-16, respectively. The numbers of *T. solium* eggs used for each dot are indicated at the right of the rows.

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. \$0.17 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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Sofia Fragoso typed the manuscript, Salvador Barbosa and Enrique Vazquez did the drawings, and Jose Aviles took the photographs. We thank Carolyn Peterson for providing DNA from the Iowa isolate of *C. parvum*.

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