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Research brief

Taenia saginata: Differential diagnosis of human taeniasis by polymerase chain reaction-restriction fragment length polymorphism assay

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Abstract

Speciation of *Taenia* in human stool is important because of their different clinical and epidemiological features. DNA analysis has recently become possible which overcomes the problems of differentiating human taeniid cestodes morphologically. In the present study, we evaluated PCR coupled to restriction fragment length polymorphism to differentiate *Taenia solium* from *Taenia saginata* eggs present in fecal samples from naturally infected patients. A different DraI-RFLP pattern: a two-band pattern (421 and 100 bp) for *T. saginata* and a three-band pattern (234, 188, and 99 bp) for *T. solium* was observed allowing the two species to be separated.. The lower detection limit of the PCR-RFLP using a non-infected fecal sample prepared with a given number of *T. saginata* eggs was 34 eggs in 2 g stool sediment. The 521 bp mtDNA fragment was detected in 8 out of 12 *Taenia* sp. carriers (66.6%). Of these, three showed a *T. solium* pattern and five a *T. saginata* pattern.

Index Descriptors and Abbreviations: DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism assay; Cestode

Keywords: Taeniasis; PCR-RFLP; Diagnosis; Fecal samples; *Taemia saginata*; *Taenia solium*

Taeniasis in humans is caused by the adult stage of *Taenia solium* and *Taenia saginata* with a worldwide distribution and also by *Taenia saginata asiatica* in Asia. Although the adult is relatively innocuous, the larval stage of *T. solium*, in contrast, can cause severe disease in man, particularly in the central nervous system (Yamasaki et al., 2004). Besides their public health importance, larval *T. solium* and *T. saginata* can infect pigs and cattle, respectively, causing economic loss principally because infected carcasses are condemned and destroyed.

Several methods based on the morphology of tapeworm proglottids have been described to differentiate between *T. solium* and *T. saginata* (Yamasaki et al., 2004). However, these require entire proglottids to be present in the sample, which reduces sensitivity (González et al., 2002). Immunodiagnosis based on coproantigen detection by enzyme-linked immunosorbent assay (ELISA) has better sensitivity but cannot differentiate between *T. solium* and *T. saginata* taeniasis (Allan et al., 2003; Deplazes et al., 1991). DNA differential diagnosis of human taeniid cestodes has been very useful and

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it includes the use of DNA probes (Chapman et al., 1995; Flisser et al., 1988; Harrison et al., 1990; Rishi and McManus, 1988), polymerase chain reaction—PCR (González et al., 2000, 2002; McManus et al., 1989; Gottestein et al., 1991; Yamasaki et al., 2004) and PCR coupled to restriction fragment length polymorphism (Mayta et al., 2000; Rodriguez-Hidalgo et al., 2002; Yamasaki et al., 2002). To date, very few articles have reported on DNA based techniques to differentiate *T. solium* from *T. saginata* in human fecal samples (Nunes et al., 2003; Yamasaki et al., 2004). In the present study, we evaluated PCR coupled to restriction fragment length polymorphism for *T. solium* and *T. saginata* differentiation in human fecal samples.

Taenia solium metacestodes were excised from the skeletal muscle of naturally infected pigs. *Taenia crassiceps* metacestodes were obtained from AJ Snell mice experimentally infected by the ORF strain of *T. crassiceps* by intraperitoneal inoculation. *Taenia saginata* proglottids and stool samples were obtained from a naturally infected patient after informed consent and approval from the Internal Ethics Committee of São Paulo State University (UNESP), Araçatuba Campus.

To evaluate the lower detection limit of taeniid mitochondrial DNA in fecal samples, a given number of *T. saginata* eggs (ranging from 8 to 17,500 eggs) were mixed with a fecal sample (2 g stool sediment) from a volunteer with no history of taeniasis. Eggs stored in 70% ethanol were counted using a plankton-counting chamber (Tsunoda and Ishii, 1971).

Twelve fecal samples were obtained from naturally infected patients after informed consent and approval from the Local Ethics Committee. Samples were stored at −20 °C until use.

Total DNA from parasite materials was obtained by phenol extraction and ethanol precipitation (Sambrook et al., 1989). Copro-DNA from fecal samples was extracted with DNAzol reagent (Invitrogen, Carlsbad, California) after sedimentation, as previously described (Nunes et al., 2003), followed by a second round of purification using the QIA quick PCR purification kit (Quiagen, Helden, Germany), before amplification.

PCR was performed with oligonucleotide primers designed from the cytochrome *c* oxidase subunit 1 gene (cox1) from human taeniid cestodes (GenBank AB066488 and AB066495) as follows: forward primer 5′ tgggttagatgttaagacggc 3′ and reverse primer 5′ aaaacacccactaaaagcaga 3′, in a volume of 50 µl of 3.75 mM each deoxynucleotide triphosphate, 2.5 mM magnesium chloride, 30 pmol each primer, 2.5 U *Taq* DNA polymerase (Invitrogen-Carlsbad, California), 5 µl 10× Invitrogen reaction buffer (Carlsbad, California) and 2 µl stool sample DNA diluted 1:2 in water or 10 ng *Taenia* metacestode DNA. DNA fragment resulting of PCR amplification was of 521 bp for both *T. saginata* and *T. solium*. The cycling conditions was: 95 °C for 5 min (initial denaturation), followed by 35 cycles at 95 °C for 60 s, 65 °C for 90 s, 72 °C for 60 s and a final extension at 72 °C for 5 min. Amplification was carried out in a PTC100 thermocycler (MJ Research, Waltham, Massachusetts) and DNA fragments were subjected to electrophoresis in 8% polyacrylamide gels stained with silver nitrate. A fecal DNA sample from a patient with no history of *Taenia* infection was used as negative control (no DNA) and DNA from *T. solium* metacestodes was included in each experiment. Each sample was tested at least twice in duplicate.

Restriction fragment length polymorphism (RFLP) was determined by using 15 U restriction endonuclease *DraI* (Invitrogen, Carlsbad, California) plus 3 µl PCR products and 2 µl enzyme buffer in a total volume of 20 µl. Tubes containing the reaction mix were incubated at 37 °C overnight. DNA was subjected to electrophoresis in 8% polyacrylamide gels and stained with silver nitrate.

In the present study, a mitochondrial DNA (mtDNA) fragment of 521 bp from *T. saginata*, *T. solium*, and *T. crassiceps* was amplified by PCR using the designed oligonucleotide primers (Figs. 1a, c, and e). After RFLP analysis with the enzyme *Dra*I, we observed different patterns for *T. saginata* and *T. solium*, which permits differentiation between the two taeniid: a two-band pattern (421 and 100 bp) for *T. saginata* (Fig. 1b) and a three-band pattern (234, 188, and 99 bp) for *T. solium* (Fig. 1d). The pattern observed for *T. crassiceps* was the same as the one observed for *T. saginata* (Fig. 1f).

We have established the lower detection limit of the PCR-RFLP using a non-infected fecal sample mixed with a given number of *T. saginata* eggs. The lower detection limit was 34 *T. saginata* eggs in 2 g stool sediment (Fig. 2k), which was higher than previously described with the use of a two step HDP2-PCR repetitive DNA amplification (Nunes et al., 2003). The same mtDNA fragment was not observed in a non-infected fecal sample (Fig. 2l). González et al. (2000) have

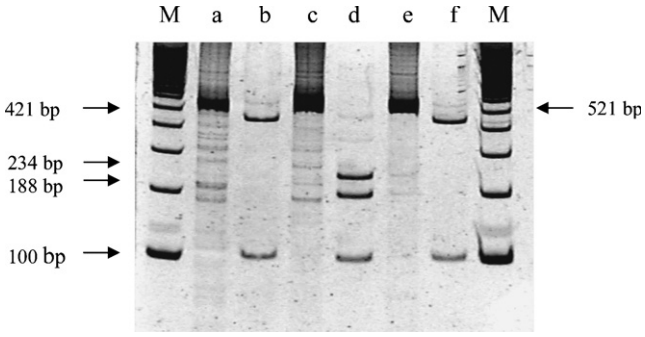

Fig. 1. Polyacrylamide gel electrophoresis (8%) silver stained from PCR fragments obtained by using genomic DNA from: *T. saginata* metacestodes (a); *T. solium* metacestodes (c); *T. crassiceps* metacestodes (e) 100 bp ladder molecular marker (M). Restriction endonuclease *Dra*I patterns obtained by digestion of PCR products from *T. saginata*, *T. solium*, and *T. crassiceps* (respectively b, d, and f).