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HELMINTHOLOGY - SHORT COMMUNICATION

Molecular characterization of the *Taenia solium* Tso31 antigen and homologous of other *Taenia* species from Peru

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Abstract

Several studies have been performed to determine specific antigens for the diagnosis of tapeworms. One of these antigens is Tso31, which is used to differentiate *Taenia solium* and *Taenia saginata* in human feces. The aim of the present work was the molecular characterization of this protein in different tapeworm specimens collected in Peru: *T. omisa* ($n = 6$), *T. hydatigena* ($n = 7$), *T. taeniaeformis* ($n = 4$), *T. pisiformes* ($n = 1$), *T. multiceps* ($n = 7$), and *T. solium* ($n = 10$). Total DNA was extracted from each proglottid using a commercial DNA kit for tissue. A nested PCR was used to amplify a fragment of the previously described oncosphere-specific protein Tso31 gene. The nested PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized after ethidium bromide staining. All nested PCR-positive products were sequenced and their sequences were compared. Of all the tapeworms analyzed, only *T. solium* and *T. multiceps* amplified the Tso31 gene. All sequences were identical for each species. Our *T. solium* Tso31 showed 100% similarity when compared with published GenBank sequences. The difference between *T. solium* and *T. multiceps* Tso31 samples was 8.1%. In conclusion, our results show that the tsol31 gene is not exclusive to *T. solium*.

Keywords *Taenia solium* · Tso31 gene · Homologous · Tapeworms

Introduction

Taenia solium is considered the most important zoonotic parasite worldwide, resulting in the taeniasis-cysticercosis pathology in humans (Gonzales et al. 2016). Human carriers of the adult *T. solium* play a key role in cysticercosis transmission (Garcia et al. 2016). A patient harboring a single adult *T. solium* parasite can release thousands of eggs into the environment, representing a great risk to themselves and to other people or pigs who are living or roaming nearby. However, pigs located geographically far from the tapeworm carrier may also show seroprevalence to *T. solium* cysticercosis (Lescano et al. 2009), suggesting that fomites or mechanical vectors may play a role in *T. solium* egg dispersion. Additionally, some insects can ingest and keep eggs of *T. solium* in their digestive tract for weeks (Gomez-Puerta et al. 2014). In this manner, the detection of parasite DNA in possible mechanical vectors may potentially elucidate a proxy for environmental contamination by *T. solium*.

Molecular evaluation of *T. solium* in environmental samples is restricted by the lack of systematic evaluations of the specificity of available primers. At present, most molecular assays focus on identifying human tapeworms and as such they do not evaluate specificity of all animal tapeworm species that may exist in the region (Flores et al. 2018), information that would be required to evaluate environmental *T. solium* contamination. This bioinformatics analysis could be partially performed using the sequences registered in GenBank. However, sequence data from other tapeworm species is quite limited.

From prior works on the serological and molecular diagnoses of taeniasis (mostly focused on discriminating

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T. solium from *T. saginata* infections), one of the most promising antigens is a 31.3 kDa excretory/secretory oncosphere antigen initially described by Verastegui et al. (2003). This protein was later used in a nested PCR assay for the specific diagnosis of taeniasis due to *T. solium* using human stool samples (Mayta et al. 2008), proving that the corresponding Tso31 gene is conserved in *T. solium* but not in *T. saginata*. However, it is not known if the Tso31 gene is homologous or conserved in other *Taenia* species. The aim of the present study was to identify and molecularly characterize the Tso31 gene or its homolog in different *Taenia* species endemic in Peru.

Material and methods

Parasitic *Taenia* spp. material Samples used in this study were proglottids from *T. omissa* ($n = 6$), *T. hydatigena* ($n = 7$), *T. taeniaeformis* ($n = 4$), *T. pisiformes* ($n = 1$), *T. multiceps* ($n = 7$), and *T. solium* ($n = 10$). All samples were provided by the Faculty of Veterinary Medicine at the Universidad Nacional Mayor de San Marcos. *T. solium* samples had been collected from parasite material expelled after treatment of human patients as part of standard of care. All cestodes from animals and humans were collected and preserved in 70% alcohol. Then, some proglottids from each tapeworm were preserved at 96% alcohol at −20 °C until DNA extraction; the rest of the tapeworm (scolex and mature proglottids) were used for microscopic identification.

DNA extraction from *Taenia* samples Alcohol-preserved tapeworm samples were dried for 10 min at room temperature. DNA was extracted using FastDNA spin kit for soil (Qbiogene Inc.®, Carlsbad, CA, USA) according to the manufacturer's protocol.

Primers In this study, a method based on a nested PCR was used to detect the *T. solium* oncosphere protein Tso31 (Mayta et al. 2008). The outer nested PCR was performed using primers F1 (5′ ATG ACG GCG GTG CGG AAT TCT G 3′) and R1 (5′ TCG TGT ATT TGT CGT GCG GGT CTA C 3′) which were predicted to amplify a 691-bp segment. The inner nested PCR was performed using primers F589 (5′ GGT GTC CAA CTC ATT ATA CGC TGT G 3′) and R294 (5′ GCA CTA ATG CTA GGC GTC CAG AG 3′) predicted to amplify a 234-bp DNA fragment. Moreover, we amplified the partial fragment of the cytochrome C oxidase I (COI) gene (~400 pb) using the primers JB3 (5′ TTT TTT GGG CAT CCT GAG GTT TAT 3′) and JB4.5 (5′ TAA AGA AAG AAC ATA ATG AAA ATG 3′) (Bowles and McManus 1994), as a control for the quality of the DNA of each sample.

PCR conditions The outer nested PCR template consisted of 0.8 μM of each primer, 2 μl of DNA, ultrapure water, and master mix (GoTaq® Green Master Mix, Promega, Madison, WI, USA) in a total volume of 25 μl. The inner nested PCR template was similar to that used for the outer nested PCR, except that 1 μl of the first nested PCR amplification was used as a DNA template. Nested PCR amplification was carried out in a thermocycler (GeneAmp 9700, Applied Biosystems, Carlsbad, CA). The first nested PCR utilized an initial denaturation step at 95 °C for 3 min, followed by 25 amplification cycles, each consisting of a denaturation step at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min. The second nested PCR was performed similar to the first, except with 40 cycles and an annealing step at 60 °C for 30 s. PCR to amplify the partial COI gene consists in 0.2 μM of each primer, 6 μl of DNA, ultrapure water, and master mix in a total volume of 50 μl. PCR amplification was carried out in a thermocycler using an initial denaturation step at 94 °C for 5 min, followed by 36 amplification cycles, each consisting of a denaturation step at 94 °C for 30 s, annealing at 50 °C for 30 s, elongation at 72 °C for 30 s, and extension at 72 °C for 10 min.

Sequencing analysis All positive samples for nested PCR were cleaned using ChromasPro software (version 2.6.4, Technelysium, South Brisbane, Australia). Then, *T. solium* Tso31 sequences were aligned and compared with those of the other tapeworms amplified using the Bioedit program (version 5.0.6, Biosoft, MO, USA). Nucleotide sequences of the partial Tso31 gene of *T. solium* and the Tso31-homologous from *T. multiceps* (Tm31) from this study were deposited into the GenBank.

Results and discussion

All samples were positive for the COI gene. Moreover, all DNA samples extracted from *T. solium* proglottids after Tso31 nested PCR gave a single amplification product of 234 bp. In addition, all seven DNA samples extracted from *T. multiceps* proglottids also amplified a product of 234 bp. Tso31 nested PCR did not amplify DNA obtained from proglottids of *T. omisa*, *T. hydatigena*, *T. pisiformis*, and *T. taeniformis*. Furthermore, Tso31 of *T. solium* study specimens (accession numbers 2155656, 2157056, 2157058, 2157059, 2157061, 2157062, 2157063, 2157065, 2157067, 2157068) was 100% identical with sequences of *T. solium* Tso31 registered in GenBank (accession number AY752889). Meanwhile, Tso31 from *T. solium* and Tm31 of *T. multiceps* (accession numbers 2152414, 2153211, 2153214, 2153216, 2153217, 2153221, 2153223) had 91.9% similarity between their nucleotide sequences.

Several assays based on DNA have been developed to differentiate *T. solium* from *T. saginata* and *T. asiatica* (Flores et al. 2018; Ng-Nguyen et al. 2017; Praet et al. 2013; Yamasaki et al. 2004). These assays were designed exclusively for use with human feces. They cannot be applied to environmental sample evaluation, such as soil, water, and mechanical vector (i.e., invertebrates and birds), because of the lack of data regarding specificity with other *Taenia* species. Detection of environmental *T. solium* contamination could be of great epidemiological importance, especially to assess the effects of control interventions (Garcia et al. 2016). This study evaluated and compared the gene sequences that encode the *T. solium* oncosphere-specific protein Tso31 and its homologs from other *Taenia* species endemic to Peru. *T. asiatica* was not included in our study because there are no human or pig cases reported in Peru. We found that nested PCR for Tso31 only amplified the samples of *T. solium* and *T. multiceps*. *Taenia* species can share homologous, but not identical, coding genes. The vaccine against porcine cysticercosis based on the homolog Tso18 *T. solium* gene proved to be as effective as the vaccine originally designed against ovine cysticercosis based on the To18 *T. ovis* gene (Gauci et al. 1998; Gauci et al. 2006). Likewise, other studies revealed that Tsa18 *T. saginata* and Tm18 *T. multiceps* homologs also showed a considerable conservation of this gene (Gauci et al. 2008; Lightowlers et al. 1996).

Since the Tso31 gene is present in *T. solium* and not in *T. saginata*, its use was recommended for differential diagnosis (Mayta et al. 2008). However, our results also show that Tso31 is present in *T. multiceps* but not in the other analyzed tapeworm species (*T. omisa*, *T. hydatigena*, *T. pisiformis*, and *T. taeniformis*). In conclusion, our findings demonstrate that the Tso31 gene is not exclusive to *T. solium* as previously believed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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