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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 PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized after ethidium bromide staining. All 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 specie. Our *T. solium* Tso31 showed 100% of 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 tso31 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 (Garcia et al. 2003). Human carriers of the adult *T. solium* play a key role in cysticercosis transmission (Garcia et al. 2016). A patient

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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 diagnosis of taeniasis (mostly focused on discriminating *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* (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 homologue 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 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 samples were preserved in 70% alcohol at room temperature until processed.

DNA extraction from *Taenia* samples:

Alcohol-preserved tapeworms samples were dried for ten minutes 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 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 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.

PCR conditions:

The outer 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 PCR template was similar to that used for the outer PCR, except that 1 µl of the first PCR amplification was used as a DNA template. PCR amplification was carried out in a thermocycler (GeneAmp 9700, Applied Biosystems, Carlsbad, CA). The first 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 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min. The second PCR was performed similar to the first, except with 40 cycles and an annealing step at 60°C for 30 sec.

Sequencing analysis:

All positive samples 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 DNA samples extracted from *T. solium* proglottids after Tso31 nested PCR gave a single amplification product of 234-bp. In addition, all eight 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) were 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). These assays were designed exclusively for use with human feces. They cannot be applied to environmental sample evaluation 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 homologues from other *Taenia* species endemic to 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 homologue 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* homologues 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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