Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular & Biochemical Parasitology 146 (2006) 101–104
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Short communication

Conservation of the vaccine antigen gene, *TSOL18*, among genetically variant isolates of *Taenia solium*☆

Charles G. Gauci[a,*], Akira Ito[b], Marshall W. Lightowlers[a]

[a] *University of Melbourne, Department of Veterinary Science, 250 Princes Highway, Werribee, Vic. 3030, Australia*
[b] *Department of Parasitology, Asahikawa Medical College, Asahikawa 078-8510, Japan*

Received 15 September 2005; received in revised form 14 October 2005; accepted 14 October 2005
Available online 8 November 2005

Keywords: *Taenia solium*; Cysticercosis; Vaccine; Gene; Genotype

Taenia solium is a cestode parasite that is transmitted between humans and pigs, and is widely distributed in many developing countries. The parasite causes cysticercosis in both hosts, which acquire the disease through direct or indirect contact with human carriers of the adult tapeworm. A serious form of the human disease, neurocysticercosis, often occurs due to *T. solium* having the propensity to encyst in neural tissue.

A recombinant vaccine has been developed which can prevent *T. solium* infection in pigs [1,2]. Use of the vaccine has the potential to interrupt the parasite's transmission to humans. The recombinant antigen, TSOL18, was cloned from the oncosphere stage of the parasite [3]. The parasite material used to isolate the cDNA encoding the TSOL18 antigen was from a *T. solium* isolate obtained from Mexico. The gene encoding TSOL18 has also been cloned and characterized [4] from *T. solium* genomic DNA of Mexican origin. The recombinant TSOL18 antigen, expressed in *Escherichia coli*, has been found to have high efficacy in preventing pig infections with *T. solium* in experimental vaccine trials conducted in Mexico, Cameroon [1] and Peru [2]. The high degree of protective immunity induced by the TSOL18 antigen suggests that vaccination may provide a practical and effective strategy to help control transmission of *T. solium* to humans and may, in future, allow eradication to become an achievable goal [2,5].

In this study, we investigate the extent of genetic polymorphism of the *TSOL18* gene in field isolates of *T. solium* from various geographical locations where the disease is prevalent. This information is useful in determining whether genetic/antigenic variability in *T. solium* would have an impact on the effectiveness of the TSOL18 vaccine if it were used as part of a disease eradication program against field-derived infection.

The DNA sequence of the mitochondrial genome of *T. solium* has previously been determined [6]. The *cytochrome c oxidase subunit 1* (*cox1*) within the mitochondrial genome has been identified as a suitable genetic marker for investigations into genetic polymorphism of *T. solium* from various worldwide geographical locations [7–9]. In previous studies, PCR amplification and DNA sequencing of the *cox1* gene from 13 isolates of *T. solium* from various geographical regions have provided evidence that indicates this parasite shows a degree of genetic variation. Two main genotypes of *T. solium* were found to exist worldwide, consisting of isolates from Asia constituting one genotype and a separate, combined genotype of isolates from both Latin America and Africa [10,11].

A total of 10 isolates of *T. solium* from Mexico, Peru, Ecuador, India, China, Indonesia, South Africa, Mozambique, Cameroon and Tanzania were included in this study (Table 1). Parasite tissues were stored in absolute ethanol prior to DNA extraction. Genomic DNA was extracted as described previously [12,13]. PCR was used to amplify the *TSOL18* gene from 24 to 135 ng *T. solium* genomic DNA using Pfx DNA polymerase (Invitrogen) under standard conditions using the following primers: 5′-GACGTTCACGACGACGAAGATG-3′ and 5′-CATTACTAACACCCTGTATTTGTATCG-3′, located in the 5′-UTR and 3′-UTR of the *TSOL18* gene, respectively. Blank PCR reactions (without genomic DNA) were carried out in parallel to rule out possible PCR contamination. PCR amplification was for 35 cycles at an annealing temperature of 60 °C. Agarose gel electrophoresis was used to confirm the presence of a 1.38 kb *TSOL18* PCR product from each of the isolates which was purified from the gel using MinElute gel extraction (QIAGEN).

Abbreviations: Cox1, cytochrome *c* oxidase subunit 1

☆ *Note:* Nucleotide sequence data reported in this paper are available in the Genbank™, EMBL and DDBJ databases under accession numbers DQ202385–DQ202386.

* Corresponding author. Tel.: +61 3 9731 2291; fax: +61 3 9741 5461.
E-mail address: charlesg@unimelb.edu.au (C.G. Gauci).

0166-6851/$ – see front matter
doi:10.1016/j.molbiopara.2005.10.014

Table 1
Sample designation, source, *cox1* genotype and identity to the *TSOL18* gene of *T. solium* isolates from various worldwide geographical locations

Sample designation	Source/geographical location	*T. solium cox1* genotype[a]	*TSOL18* identity[b]
Mexico	Adult, Mexico	Latin American/African	100
Peru	Cysticercus, Peru	Latin American/African	100
Ecuador	Cysticercus, Ecuador	Latin American/African	100
India	Cysticercus, Vellore, India	Asian	100
China	Adult, Yunnan, PR China	Asian	100
Indonesia	Adult, Papua, Indonesia	Asian	100
SthAfrica	Cysticercus, South Africa	Latin American/African	100
Mozambique	Cysticercus, Mozambique	Latin American/African	100
Cameroon	Adult, Cameroon	Latin American/African	100
Tanzania	Cysticercus, Tanzania	Latin American/African	100

[a] *T. solium* genotype designation in accordance with Nakao et al. [11].
[b] Percent identity to the *TSOL18* gene of the proven Mexican isolate from which the cDNA expressing the TSOL18 vaccine was cloned by Gauci et al. [3].

The *cox1* genotype for each of the 10 *T. solium* isolates was determined by PCR amplification of a 396 bp portion of *cox1* using the following primers at an annealing temperature of 50 °C: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′ and 5′-TAAAGAAAGAACATAATGAAAATG-3′ [7]. PCR and purification conditions were as described above.

Direct DNA sequencing was performed on the purified *TSOL18* and *cox1* PCR products using an ABI PRISM BigDye

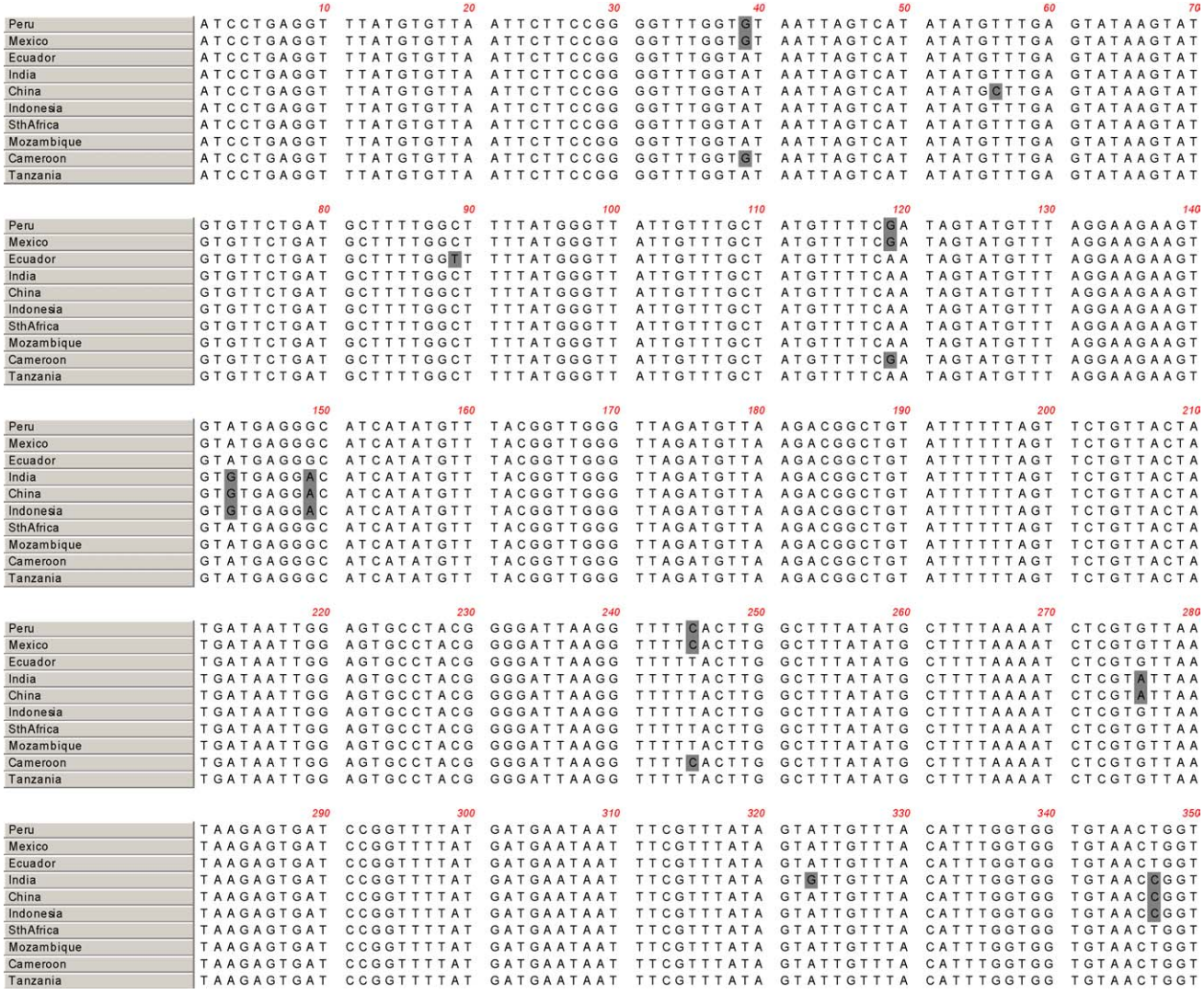

Fig. 1. DNA sequence alignment of *cox1* from *T. solium* isolates obtained from different geographical locations. Variant nucleotides are shaded.