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Short communication

## Conservation of the vaccine antigen gene, *TSOL18*, among genetically variant isolates of *Taenia solium*<sup> $\Leftrightarrow$ </sup>

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*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

 $<sup>^{\</sup>Rightarrow}$  *Note:* Nucleotide sequence data reported in this paper are available in the Genbank<sup>TM</sup>, EMBL and DDBJ databases under accession numbers DQ202385–DQ202386.

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Table 1

Sample designation	Source/geographical location	T. solium cox1 genotype <sup>a</sup>	TSOL18 identity <sup>b</sup> 100	
Mexico	Adult, Mexico	Latin American/African		
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	
anzania Cysticercus, Tanzania		Latin American/African	100	

<sup>a</sup> *T. solium* genotype designation in accordance with Nakao et al. [11].
 <sup>b</sup> 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

	10	20	30	40	50	60	70
Peru	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTGT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
Mexico	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	G G T T T G G T <b>G</b> T	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
Ecuador	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
India	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
China	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGCTTGA	GTATAAGTAT
Indonesia	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
SthAfrica	ATCCTGAGGT	TTATGTGTTA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
Mozambique	ATCCTGAGGT	TTATGTGTTA	A T T C T T C C G G A T T C T T C C G G	G G T T T G G T A T G G T T T G G T <b>G</b> T	A A T T A G T C A T A A T T A G T C A T	ATATGTTTGA	GTATAAGTAT
Cameroon	ATCCTGAGGT				AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
Tanzania		TIATOTOTIA	ATTCTTCCGG	GGTTTGGTAT	AATTAGTCAT	ATATGTTTGA	GTATAAGTAT
	80	90	100	110	120	130	140
Peru	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCGA	TAGTATGTTT	AGGAAGAAGT
Mexico	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCGA	TAGTATGTTT	AGGAAGAAGT
Ecuador	GTGTTCTGAT	GCTTTTGG <b>T</b> T	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
India	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
China	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
Indonesia	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
SthAfrica	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
Mozambique	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
Cameroon	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCGA	TAGTATGTTT	AGGAAGAAGT
Tanzania	GTGTTCTGAT	GCTTTTGGCT	TTTATGGGTT	ATTGTTTGCT	ATGTTTTCAA	TAGTATGTTT	AGGAAGAAGT
2	150	160	170	180	190	200	210
Peru		ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
Mexico	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
Ecuador	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
India China	G T G T G A G G A C G T G T G A G G A C	A T C A T A T G T T A T C A T A T G T T	T A C G G T T G G G T A C G G T T G G G	T T A G A T G T T A T T A G A T G T T A	A G A C G G C T G T A G A C G G C T G T	A T T T T T T A G T A T T T T T T A G T	Т С Т G Т Т А С Т А Т С Т G Т Т А С Т А
Indonesia	GTGTGAGGAC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT		
SthAfrica	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	A T T T T T T A G T A T T T T T T A G T	Т С Т G Т Т А С Т А Т С Т G Т Т А С Т А
Mozambique	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
Cameroon	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
Tanzania	GTATGAGGGC	ATCATATGTT	TACGGTTGGG	TTAGATGTTA	AGACGGCTGT	ATTTTTAGT	TCTGTTACTA
	220	230	240	250	260	270	280
Peru	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTCACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTGTTAA
Mexico	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTCACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTGTTAA
Ecuador	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTGTTAA
India	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTATTAA
China	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	CTTTTAAAAT	СТСБТАТТАА
Indonesia	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	СТТТТААААТ	CTCGTGTTAA
SthAfrica	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	СТТТТААААТ	СТСБТБТТАА
Mozambique	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTGTTAA
Cameroon		AGTGCCTACG	GGGATTAAGG		GCTTTATATG	СТТТТААААТ	CTCGTGTTAA
Tanzania	TGATAATTGG	AGTGCCTACG	GGGATTAAGG	TTTTTACTTG	GCTTTATATG	CTTTTAAAAT	CTCGTGTTAA
	290	300	310	320	330	340	350
Peru	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
Mexico	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
Ecuador	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
India	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTGTTGTTTA	CATTTGGTGG	TGTAACCGGT
China	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACCGGT
Indonesia	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACCGGT
SthAfrica	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
Mozambique	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
Cameroon	TAAGAGTGAT	CCGGTTTTAT	GATGAATAAT	TTCGTTTATA	GTATTGTTTA	CATTTGGTGG	TGTAACTGGT
Tanzania		CCGGTTTTAT		TTCGTTTATA		CATTTGGTGG	TGTAACTGGT
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Fig. 1. DNA sequence alignment of cox1 from T. solium isolates obtained from different geographical locations. Variant nucleotides are shaded.

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