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ORIGINAL PAPER

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# *Taenia solium* DNA is present in the cerebrospinal fluid of neurocysticercosis patients and can be used for diagnosis

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**Abstract** Neurocysticercosis is the most frequent parasitic infection of the CNS and the main cause of acquired epilepsy worldwide. Seizures are the most common symptoms of the disease, together with headache, involuntary movements, psychosis and a global mental deterioration. Absolute diagnostic criteria include the identification of cysticerci, with scolex, in the brain by MRI imaging. We demonstrate here, for the first time, that *T. solium* DNA is present in the cerebrospinal fluid of patients. The PCR amplification of the parasite DNA in the CSF enabled the correct identification of 29/30 cases (96.7 %). The PCR diagnosis of parasite DNA in the CSF may be a strong support for the diagnosis of neurocysticercosis.

**Key words** PCR · CSF · neurocysticercosis · *Taenia solium* · liquor

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## Introduction

When the adult *Taenia solium* cestode infests the human intestine, taeniasis develops. This infection is usually asymptomatic, but the detection of the parasite is important because of the risk of spreading eggs. When adequate sanitary conditions are not available, the eggs may contaminate food or water and their ingestion enables the development of the larvae, named cysticercus, in humans or pigs. These larvae enter the circulation, and lodge in the tissues, usually the brain or muscles. When the cysts are located in the central nervous system (CNS), they cause neurocysticercosis (NC), the most common parasitic infection of the CNS. The clinical presentations are pleomorphic and depend on the stage, number and location of cysts in the nervous system. Seizures are frequent and can reach 50 to 80 % of patients with parenchymal brain cysts or calcifications (Chopra et al. 1981; DelBrutto et al. 1992). Psychiatric symptoms have been frequently reported, reaching 65.8 % of the cases (Forlenza et al. 1997). NC must be considered in the differential diagnosis of seizures and a wide variety of disorders, particularly in endemic areas.

NC is globally spreading due to the increase of migration, immigration, tourism and globalization (Earnest et al. 1987; Wallin et al. 2004). It is estimated that globally 50 million people are infected with the taeniasis/cysticercosis complex and 50,000 die from cysticercosis annually (CDC 1993), whereas a much higher number present other types of neurological damages (Ito et al. 2003). It is believed that, due to the lack of reli-

able diagnostic tests, the prevalence of NC is underestimated in many areas (Ito and Craig 2003).

Whereas the high costs of magnetic resonance images, nowadays the most accurate method of diagnosis, may be prohibitive especially in endemic regions, the interpretation of the multiple immunologic tests available is often difficult due to lack of specificity or sensitivity. Considering the high specificity and sensitivity usually attained by DNA-based diagnostic tests, we evaluated the presence of cysticercus-DNA in the cerebrospinal fluid (CSF) of patients. This new approach may provide additional support to define diagnosis, since imaging procedures are costly and have scarce availability in areas where most of the affected population is located.

## Materials and methods

The presence of *T. solium* DNA was investigated by using primers (TSF 5'-CAGGGTGTGACGTCATGG-3' and TSR 5'-GCTAGGCAACTGGCCTCCT-3') directed against pTsol9, a highly repetitive element of the parasite genome (GenBank accession code: U45987). The PCR amplification protocol used here is a two-step reaction without the primer-extension step. Reactions were performed in a MJ Research PTC-100 Thermal cycler (Watertown, MA) in a final volume of 10 µl, containing 30 mM Tris-HCl (pH 8.8), 15 mM KCl, 3 mM $MgCl_2$, 2 pmol of each oligo, 1.88 µM of dNTPs (Invitrogene, USA) and 1 U of Taq DNA polymerase (New England, Biolabs; Beverly, MA). In the first cycle, DNA was denatured at 95 °C for 3 min, followed by 38 amplification cycles of denaturing at 95 °C for 45 s and primer annealing at 56 °C for 1 min. Five microliters of PCR amplification products were evaluated in silver-stained polyacrylamide gels (Sanguinetti et al. 1994).

Sensitivity of *T. solium* DNA detection was evaluated with serial dilutions of DNA extracted from mature proglottids of *T. solium* and quantified using spectrophotometric reads at 260 nm. This experiment was repeated 3 times, using distinct DNAs, and the same detection limits were consistently obtained. Specificity of DNA amplification was demonstrated by the absence of amplification in human DNA as well as in DNA derived from related parasite species (*Taenia saginata, Taenia crassiceps* and *Schistosoma mansoni*). The specificity of the amplification products was confirmed by direct sequencing of the PCR products using an ABI3100 DNA sequencer and dideoxi-terminators (Applied Biosystems, Foster City, USA – data not shown).

Analyses in clinical samples were conducted in material derived from 30 consecutive patients with NC, all fulfilling Del Brutto's criteria for NC diagnosis (Del Brutto et al. 2001) and 10 non-NC controls (meningeal carcinomatosis, intramedullar tumor, exogenous intoxication, tensional headache). Epilepsy was present in 29 patients; intracranial hypertension in one. In 26 patients vesicles were lodged in encephalic parenchyma; cisternal forms were found in two, ventricular form in one and mixed form in one other patient. Informed consent was obtained from all subjects, after the approval of the study by the ethics committees of the institutions involved. PCR was performed in CSF collected after lumbar puncture prescribed for clinical investigations in all subjects. When higher volumes of CSF are used for PCR, enzyme inhibitors may preclude DNA-amplification that can also fail when DNA is not present in sufficient amounts. To circumvent problems related to enzyme inhibitors or too diluted DNA in CSF, we have purified CSF samples using the Perfect gDNA Blood kit (Eppendorf Scientific, Westbury, USA), concentrating the DNA extracted from 100 µl of CSF in 25 µl of water. All PCR amplifications were tested in triplicates for pure and extracted samples (1 µl, 3 µl and 5 µl for each condition), resulting in 18 reactions per DNA sample.

## Results

Using the pair of oligonucleotide primers described here, a band of 120 bp, representing the amplification of one repeat unit, was obtained. When higher amounts of parasite genomic DNA were used (as can be seen in Fig. 1), the amplification of 2 and 3 repeat-units (bands of 278 and 436 bp, respectively) could also be observed. Serial DNA dilutions indicated a high sensitivity of the test, and as shown in Fig. 1, as little as 10 fg of genomic DNA could be amplified. This limit was consistently achieved in different evaluations, suggesting this to be the minimal amount of DNA template to give consistent amplifications. The specificity of the primer pair used here was demonstrated by the absence of amplification of this element when DNA derived from other *Taenia* species, from a closely-related parasite (*S. mansoni*) or from human were used as template (data not shown). The identity of the amplicon was confirmed by sequencing the PCR products and comparing with public DNA databases.

When pure CSF samples from patients (volumes of 1, 3 and 5 µl) were used directly as PCR templates, specific amplicons were obtained for the majority of the patients (20/30–67 %). When purified/concentrated samples, derived from 100 µl of CSF were used (1 to 5 µl), we observed that of the 30 tested samples, only one did not show the presence of *T. solium* DNA, resulting in a detection rate of 96.7 %. Amplification products derived from neat or from purified CSF, from the same patients, are shown in Fig. 2. No noticeable reduction in the specificity was seen after CSF purification and NC negative

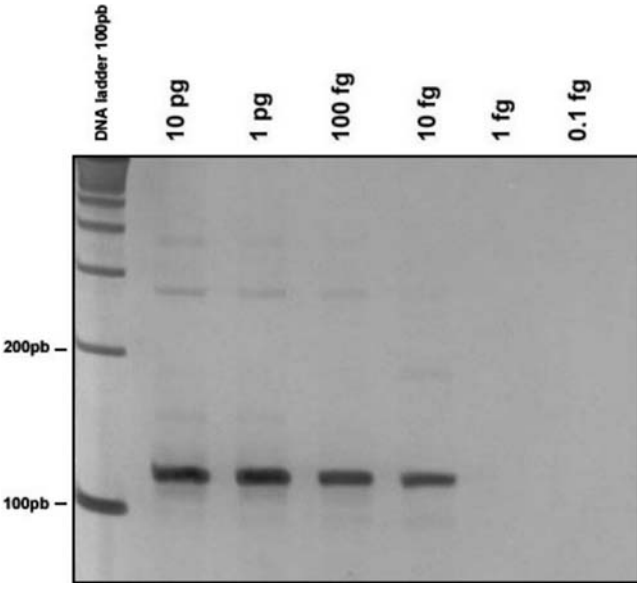


**Fig. 1** Sensitivity of amplification was determined by using serial dilutions of *Taenia solium* DNA. Shown is the amplification conducted using 10x dilutions of DNA between 10 fg and 0.1 fg. The limit of detection was repeatedly determined as 10 fg of genomic DNA. Five microliters of amplification products were evaluated in 6 % silver-stained polyacrylamide gels

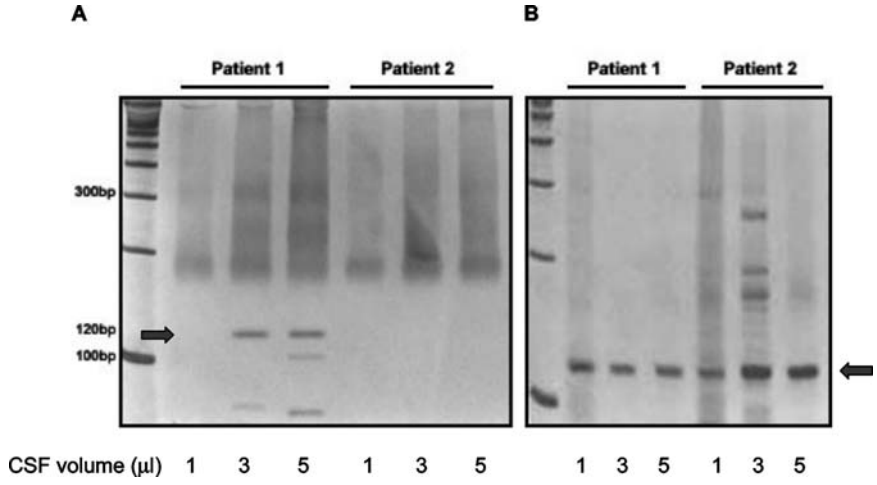


**Fig. 2** Evaluation of *Taenia solium* DNA in the CSF of two infected patients. Amplification was conducted for CSF samples of patients 1 and 2 using 1, 3 or 5 µl of CSF directly as a template for PCR amplification (**A**) or using 1, 3 or 5 µl of DNA extracted from CSF (**B**). Amplification products were evaluated in 6 % silver-stained polyacrylamide gels. Specific bands are pointed by black arrows

controls remained negative for all replicates in all template dilutions. To our knowledge, this is the first demonstration of *T. solium* DNA in the CSF of NC patients.

## Discussion

Clinical manifestations of NC are variable and unspecific, depending on the number, localization, morphological type and developmental stage of the cysticerci as well as the immune response of the host (Garcia et al. 2003). Neurological manifestations commonly include seizures and intra-cranial hypertension. Psychiatric symptoms have been frequently reported; and their severity has been correlated with treatment of neurocysticercosis, associated with an increase in CNS inflammation. Forlenza et al. reviewed 38 cases of NC and found psychiatric illness and cognitive decline in 65.8 % and 87.5 % of cases, respectively. Depressive disorders, found in 52.6 %, were the most frequent non-cognitive psychiatric illness and psychosis was seen in 14.2 %. Progression of disease and intra-cranial hypertension correlated with higher levels of psychiatric comorbidity. This variety of symptoms, which may also include headache, convulsions and cerebral sequels of long-term inflammation, which can lead to hydrocephalus, dementia, and death, contributes to the complexity of the clinical diagnosis and reinforces the need of alternative diagnostic tools.

Nowadays, absolute diagnostic criteria of NC include the identification of cysticerci, with scolex, in the brain by MRI imaging. In view of its high positive predictive value, MRI is considered as the best method for diagnosis. However, MRI may fail to find image of cysticercus in degeneration, by at least 14 months (Garcia et al. 2003), resulting in a surprising low negative predictive value. Immunologic tests for antibodies detection in NC diagnosis are clearly not sufficient to confirm nor to discard the infection: a) EITB in the serum has a sensitivity ranging from 14 % in cases with one single active lesion to more than 90 % in cases of more than one active cyst (Sanchez et al. 1999); b) ELISA in CSF may present high sensitivity (95.6 %) and specificity (100 %), as described in cases with inflammation. However, CSF specific immunocomplexes may lead to false-negative results in cases with low antigen concentration (Bueno et al. 2000) and cross-reaction may occur with antibodies against other parasites (Carpio 2002).

It is important to point out that not always the patient who presents greater number of cysts will have a standard of stronger amplification: a single active cyst may yield strong amplifications, detected with 1 µl of neat CSF, while patients presenting more active cysts can present less intense amplification profiles. Moreover, PCR was positive in the two NC cases, which were consistently negative for both antigen and antibodies detection, suggesting a better diagnostic performance of PCR in some cases. Further samples should be assayed to have a better picture of its performance. The reason of PCR failure in detecting one NC case, previously diagnosed by MRI and CSF antibodies, is not clear. It should be noted that this patient was the single case presenting *Cysticercus racemosus* in CSF space, an intense meningeal reaction, and multiple parenchymal calcified cysts.

The PCR described here amplifies a repetitive element of the parasite genome, described in 1995 by Chapman et al. These authors identified this repetitive element while screening repetitive genomic elements that could differentiate eggs of Taenia species in dotblot assays. In our analysis, this genomic region was used in PCR diagnosis for the first time. The use of this repetitive genomic element enabled the detection of 10 fg of DNA (3 % of the parasite-genome content of a single diploid cell), resulting in a high sensitivity test (96.7 %) with total specificity. It is a quick and easy assay and has a cost that enables its routine use, even in endemic areas. The demonstration that parasite-DNA is present in the CSF of NC patients may have an important impact in the diagnosis of the disease. The high detection rate, absence of unspecific amplifications, simplicity and the

low cost of the amplification reaction may encourage the adoption of PCR as an additional diagnostic support for this disease.

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