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Confirmation and Follow-Up of Neurocysticercosis by Real-Time PCR in Cerebrospinal Fluid Samples of Patients Living in France▽

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Neurocysticercosis diagnosis is based on a combination of clinical, epidemiological, radiological, and immunological findings. We describe a real-time PCR assay for the confirmation of neurocysticercosis diagnosis in cerebrospinal fluid. The assay, tested on samples from nine patients living in France and diagnosed with neurocysticercosis, had a detection rate of 83.3% and 100% specificity.

Neurocysticercosis (NCC) is endemic in developing countries (8). Epilepsy is the most common clinical presentation, although some subjects remain asymptomatic or present a wide variety of disorders, including psychosis (10) and meningoencephalitis (7). Brain imaging is crucial for the diagnosis of NCC. Computed tomography (CT) may show direct signs (live or calcified cysts) or indirect signs (deformation of the ventricles by a cyst, ventricular dilation, or hydrocephalus). However, except in end-stage calcified disease, CT is less sensitive than magnetic resonance imaging (MRI) for detecting small cysts. MRI is thus the best imaging tool, capable of showing different developmental stages of *Taenia solium* larvae, reactional edema, and ependymitis (7). Routine laboratory tests are of little value. Blood and cerebrospinal fluid (CSF) eosinophilia is inconsistently present (4). Detection of antibodies against *T. solium* in CSF can be useful: enzyme-linked immunosorbent assay (ELISA) shows high sensitivity, while immunoblotting has high specificity (9). Detection of serum antibodies is a less invasive alternative, and its sensitivity rivals that of detection in CSF. Formerly, the only definitive diagnostic method was direct pathological examination of excised cysts (5), but detection of *T. solium* DNA in CSF by conventional PCR has been reported to show high sensitivity and good specificity in patients from Latin America (1, 9). Nevertheless, in developing countries, diagnosis typically rests on serum *T. solium* antibody detection with imaging.

We developed a real-time TaqMan PCR to detect *T. solium* DNA in CSF. CSF and serum samples were obtained from nine patients with NCC diagnosed in Paris hospitals (Bichat-Claude Bernard, Saint Louis, Cochin-Port Royal, and Sainte Anne) between 2004 and 2011 and selected on the basis of Del Brutto's diagnostic criteria (clinical, imaging, immunologic, and epidemiologic) (5). Antibodies against *T. solium* were detected in CSF and serum using an immunoblot cysticercosis Western blot IgG kit (LDBio Diagnostics, France). Negative controls consisted of 15 CSF samples found positive for other central nervous system infections (*Toxoplasma gondii*, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, human immunodeficiency virus, herpes simplex viruses 1 and 2, enterovirus, JC virus, *Streptococcus pneumoniae*, *Escherichia coli*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus agalactiae*, and *Staphylococcus aureus*), 9 microbial cultures discharged into phosphate-buffered saline (*Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Propionibacterium* sp., *Fusobacterium* sp., *Bacteroides fragilis*, *Bacillus* sp., *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigatus*), and proglottids of 7 isolates of *Taenia saginata*, another member of the Cestoda class, related to *T. solium*. The patients provided informed consent before testing of samples.

DNA was extracted from 100 μl of serum, CSF, and microbial culture suspensions by using the biological fluid protocol of the QIA Amp DNA minikit (Qiagen) and was eluted in 100 μl of sterile distilled water. One hundred microliters of sterile water was also extracted and used as a negative extraction control. DNA was extracted from *T. saginata* proglottids, as previously described (11). *T. solium* DNA amplification targeted the pTsol9 repetitive element of the parasite's nuclear genome (GenBank accession number U45987) (3), using TaqMan probe detection on an ABI Prism 7000 instrument (Applied Biosystems). The PCR assay was performed in 25 μl, including 5 μl of DNA; 0.2 μM (each) primer (TSF, 5′-CAG GGTGTGACGTCATGG-3′; TSR reverse complement, 5′-A GGAGGCCAGTTGCCTAGC-3′), as described by Almeida et al. (1); 0.4 μM locked nucleic acid-substituted hydrolysis probe (5′-6-carboxyfluorescein-AGGCTG[+T][+C][+C]TTT GCCGT-black hold quencher-3′) designed in our laboratory; and 1× TaqMan universal master mix with uracil *N*-glycosylase (Applied Biosystems). The assay began at 50°C for 2 min, followed by 95°C for 10 min and 45 amplification cycles of 95°C

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TABLE 1. Clinical, radiological, immunological, and real-time PCR results for patients with NCC

Patient group and no.	Clinical signs	Cerebral radiology result	Epidemiology	Immunology result[a]	Real-time PCR result (C_T[b])
Definitive NCC[c]					
P1	Psychiatric and memory disorders, spatial disorientation, aphasia	3 IP[n] lesions	Africa, 36 yr ago	Serum positive/CSF positive	Serum negative/CSF positive (36.38)
P2	Headache, neck ache, nausea, vomiting	Multiple intraventricular lesions, ventricular dilatation, foramen of Monro obstruction	Central Africa, 21 yr ago	Serum positive/CSF positive	Serum negative/CSF positive (34.40)
P3	Headache, nausea, vomiting	IP calcifications, hydrocephalus, aqueduct of Sylvius obstruction	Haiti, 7 yr ago	Serum positive/CFS positive	Serum negative/CSF_1 negative, CSF_2[d] positive (32.92)
P4	Epilepsy, neuralgia, paresthesia	IP calcifications, hydrocephalus, arachnoiditis	Haiti, 22 yr ago	Serum positive/CSF positive	Serum negative/CSF[e] positive (31.60)
P5	Epilepsy, headache, vomiting	2 IP lesions, chronic meningitis	Cape Verde, 1 yr ago	Serum positive/CSF positive	Serum negative/CSF_1 positive (29.3), CSF_2[f] positive (21.25), CSF_3[g] positive (23.32)
P6	Epilepsy, confusion	Multiple IP lesions, hydrocephalus, vascularitis	Cape Verde, 20 yr ago	Serum positive/CSF positive	Serum negative/CSF_1 positive (26.15), CSF_2[h] positive (26.66), CSF_3[i] positive (28.01), CSF_4[j] positive (30.59), CSF_5[k] positive (29.65), CSF_6[l] positive (32.33)
Probable NCC[c]					
P7	Headache, nausea, memory disorders, dysphasia, spatial disorientation, muscular cysticercosis	IP calcifications, parasagittal meningioma, then hydrocephalus, meningoencephalitis	Madagascar, 34 yr ago	Serum positive/CSF positive	Serum negative/CSF_1 positive (39.20), CSF_2[m] positive (30.99)
P8	Headache, epilepsy	4 IP lesions	Cape Verde, 2 mo ago	Serum positive/CSF positive	Serum negative/CSF negative
P9	Headache, epilepsy	1 IP lesion	India, 6 yr ago	Serum positive/CSF negative	Serum negative/CSF negative

[a] Immunoblotting was performed with the cysticercosis Western blot IgG kit (LDBio Diagnostics, France).
[b] C_T, cycle threshold.
[c] NCC was categorized as definitive or probable according to the diagnostic criteria of Del Brutto et al. (5).
[d] CSF drawn after external ventricular derivation (fluid derived via a ventriculoperitoneal shunt), 3 days after the first sample.
[e] CSF drawn after external peritoneal derivation.
[f] CSF drawn 3 months after the first sample.
[g] CSF drawn 5 months after the first sample.
[h] CSF drawn 1 month after the first sample.
[i] CSF drawn 3 months after the first sample.
[j] CSF drawn 3 1/2 months after the first sample.
[k] CSF drawn 3 3/4 months after the first sample.
[l] CSF drawn 4 months after the first sample.
[m] CSF drawn 38 months after the first sample.
[n] IP, intraparenchymal.

for 15 s and 60°C for 1 min. Each DNA extract was tested pure and diluted 10-fold. Each PCR run included a negative extraction control, a negative PCR control (a DNA extract previously found to be negative), and a positive PCR control (a DNA extract from a patient sample previously found to be positive). Samples positive by real-time PCR were tested by DNA amplification and double-strand sequencing of the Cestoda mitochondrial cytochrome oxidase subunit I (COI) gene with primers JB3 and JB4.5 (2) as previously described (11). Amplimers produced by real-time PCR were sequenced if samples were PCR negative with primers JB3 and JB4.5. The sequences were aligned and compared with GenBank reference sequences using BLAST searches.

Six patients had a definitive diagnosis of NCC, while three had probable diagnoses. The patients received antiparasitic treatment and corticosteroids, combined in some cases with antiepileptic treatment. All 31 negative controls and negative extraction and PCR controls were negative in the real-time PCR assay. Nine sera and 18 CSF samples were drawn from the nine patients with NCC. All the sera were negative by real-time PCR. Fifteen of the 18 CSF samples were positive, confirming the diagnosis of NCC in seven of the nine patients (six definitive diagnoses and one probable diagnosis) (Table 1). The specificity of the real-time PCR assay was 100%. Its detection rate was 0/9 (0%) in serum samples, 15/18 (83.3%; 95% confidence interval [CI], 58.6% to 96.4%) in CSF samples, and 13/14 (92.9%; 95% CI, 66.1% to 99.8%) in CSF samples from patients with definitive NCC. All the sera were obtained at the time of diagnosis. CSF was obtained at the time of diagnosis from each of the nine patients and during follow-up after antiparasitic treatment from four patients (nine samples) (Table 1). One patient (P3) had two CSF samples drawn with a 3-day interval, before and after external ventricular derivation (fluid derived via a ventriculoperitoneal shunt). The first was negative by PCR, but the second was positive. In the other patients (P5, P6, and P7), all samples were positive, whatever the maximal sampling interval (5, 4, and 38 months, respectively). Parasite DNA amplifications (expressed as cycle threshold [C_T]) were compared in CSF extracts from these patients in the same run, and differences of more than 3 PCR cycles (1 log) were considered significant. Parasite DNA load increased between the first and last CSF samples in two patients (P5 and P7), while it decreased in the third patient (P6). DNA sequencing of a region of the COI gene confirmed the presence of *T. solium* DNA in the CSF of six of the seven patients (P1, P3, P4, P5, P6, and P7). Sequencing of the pTsol9 amplimer confirmed *T. solium* DNA amplification in the CSF of the last patient (P2).

T. solium larvae are reported to live for no more than 7 years in their host (4). Our patients' clinical manifestations included seizures, psychiatric disorders, focal neurological deficits, meningitis, and meningoencephalitis. Cerebral imaging and serological tests suggested NCC, but most of the patients had traveled to tropical countries more than 7 years before symptom onset (Table 1). Infection via wild boar meat in France was a possibility, but autochthonous cysticercosis is very rare in France (6) and the patients' parasitological stool examinations were negative. The real-time PCR assay on CSF confirmed NCC suspected on the basis of cerebral imaging and seropositivity in patients living outside regions of endemicity, thus avoiding the need for brain biopsy. In regions of endemicity, the difficulty in NCC diagnosis should be less the differentiation of definitive or probable cases than the differentiation of suspected patients from those who have single-lesion NCC. This real-time PCR CSF assay might also be of benefit for these patients, but further studies would evaluate its sensitivity in single-lesion NCC cases.

We found that parasite DNA could persist in CSF for several months, even after antiparasitic treatment, and sometimes correlate with the persistence of symptoms, suggesting that the larvae may not be totally eliminated by the treatment. We found that parasitic DNA load in CSF could either increase or decrease after treatment. The increase in DNA could be related to release of parasitic materials in CSF following larval lysis by antiparasitic treatment. This suggests that serial analysis of parasitic load in CSF may not be useful for disease or treatment follow-up, although further studies are needed. We found that obstruction of CSF flow could lead to a false-negative CSF PCR result and that CSF derivation led to the release of parasite DNA. PCR was negative in the patients' serum, but the number of patients as well as the serum sample volume is small, so further studies are needed to confirm this result.

The real-time CSF PCR can quickly confirm cases of NCC suggested by clinical, imaging, immunologic, and epidemiologic features. It can also detect the persistence of *T. solium* DNA in the brain after many years.

Nucleotide sequence accession numbers. The nucleotide sequences were deposited in GenBank under accession numbers JN084220 to JN084225 and JN091096.

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