Unexpected Diagnosis of Cerebral Toxoplasmosis by 16S and D2 Large-Subunit Ribosomal DNA PCR and Sequencing

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The protozoan parasite *Toxoplasma gondii* causes severe opportunistic infections. Here, we report an unexpected diagnosis of cerebral toxoplasmosis. *T. gondii* was diagnosed by 16S and D2 large-subunit (LSU) ribosomal DNA (rDNA) sequencing of a cerebral biopsy specimen and confirmed by *T. gondii*-specific PCR and immunohistochemistry. The patient was later diagnosed with HIV/AIDS.

CASE REPORT

A 65-year-old man presented at a local hospital with confusion, behavioral changes, and convulsions. The patient was a retired farmer, was previously healthy, and was not taking any medications. He had returned from a holiday in Thailand 5 months earlier. Contrast magnetic resonance imaging (MRI) of the brain revealed a ring-enhancing lesion (18 by 16 mm) in the left occipital lobe, with surrounding edema (Fig. 1). The patient was treated with an antiepileptic drug (levetiracetam) and transferred to the Department of Neurosurgery on suspicion of a cerebral abscess.

On admission, the patient was afebrile; however, he was confused and demonstrated impaired memory and reading ability. Initial laboratory tests were normal, including those for infection. Biopsy specimens (guided by neuronavigation) were taken from the lesion. No pus was visible. Neither cell culture nor light microscopy after Gram and methylene blue staining revealed the presence of microorganisms. Histopathological examination of the biopsy sections showed necrosis, with no signs of abscess or malignancy. *Toxoplasma gondii* was not suspected, and specific staining was not performed at this point. Empirical antibiotic treatment with meropenem, fusidic acid, and metronidazole was initiated, but the patient did not improve. A contrast computed tomography (CT) scan performed 2 weeks later showed progression of the lesions. Subacute surgery was performed, and a 30- by 30-mm section of tissue was removed *in toto*. Microbiological and histopathological findings related to the excised tissue were similar to those for the initial biopsies. The patient was prescribed prednisolone and cefuroxime; however, the neurological symptoms progressed, and the patient had difficulty in performing routine tasks.

Five days postsurgery, the patient was transferred to the Department of Infectious Diseases for further investigation. A more detailed patient history revealed that he had in fact made several trips to Thailand to visit his Thai girlfriend, and therefore an HIV test was performed. Clinical examination of the patient revealed confusion, poor memory, and no awareness of his current condition. Total and differential white blood cell count and C-reactive protein (CRP) level remained within the normal range. Cefuroxime was replaced with meropenem, and because the cognitive symptoms regressed over the following days, the patient was weaned off prednisolone.

We requested 16S and D2 large-subunit (D2LSU) ribosomal DNA (rDNA) sequencing of one of the initial biopsy specimens to test for the presence of bacteria and fungi, respectively. Both analyses showed a high average consensus quality value, with specimen scores of 37 for bacteria and 42 for fungi (values above 30 indicate high quality), but poor percentage matches for bacterial and fungal species (59% and 60%, respectively). However, comparing the rDNA sequences with sequences in the National Center for Biotechnology Information nucleotide (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) using BLAST (Basic Local Alignment Search Tool) unexpectedly revealed 99% (16S) and 100% (D2LSU) matches with *T. gondii* sequences. rDNA sequencing was performed as follows. DNA was purified from a biopsy sample using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A 500-bp region from the 16S rRNA gene and an approximately 300-bp region of D2LSU rRNA were amplified by PCR using the Fast MicroSeq 500 16S rDNA and MicroSeq D2LSU rDNA identification kits, respectively (Applied Biosystems, Warrington, United Kingdom), and sequenced on a 3130xl genetic analyzer (Applied Biosystems, Carlsbad, USA) according to the manufacturer's instructions. Subsequently, sequence data were analyzed using MicroSeq ID 16S rDNA 500 Library v2.2 and MicroSeq ID Fungal Gene Library v2.0 software (Applied Biosystems, Carlsbad, CA, USA) (1), resulting in the aforementioned percentage matches.

To confirm a diagnosis of *T. gondii* infection, we performed *T. gondii*-specific PCR (TaqMan-based PCR targeting the 529-bp DNA repeat element; GenBank [AF487550](https://www.ncbi.nlm.nih.gov/nuclot/AF487550) [2]) of the initial biopsy

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FIG 1 MRI showing a lesion in the left occipital lobe.

specimens, along with histological examination using a *T. gondii*-specific antibody (a rabbit polyclonal anti-*T. gondii* antibody [BioGenex, Fremont, CA, USA]) and the BenchMark Ultraview detection kit (3). The diagnosis was supported by positive serological tests for toxoplasma (*T. gondii* IgG positive/IgM negative). The patient was also positive for HIV (HIV1 + 2 [Combo, Architect, Abbott] and anti-HIV1+II [Vitros Ortho, Clinical Diagnostics]), HIV1 RNA (the Procleix system), and antibodies to HIV-1 (INNO-LIA HIV1/II assay; Innogenetics/Fujirebio); he also had a CD4 count of 20 cells/ μ l (normal range, 390 to 1,700 cells/ μ l), which is indicative of severe immunosuppression. The results were further validated using a commercial strain of *T. gondii* (ATCC 40050; strain TS-4) using the method described above. The results were comparable with those obtained using the MicroSeq D2LSU and MicroSEQ16S rDNA identification kits.

The patient was treated with sulfadiazine and pyrimethamine to combat toxoplasmosis. He also received antiviral combination therapy against HIV. His clinical and paraclinical condition (contrast CT scan and immune parameters) improved markedly thereafter.

Here, 16S and D2LSU rDNA sequence analysis provided the diagnostic clue to cerebral toxoplasmosis, although these investigations are usually aimed at conditions with suspected bacterial or fungal etiologies. The diagnosis was subsequently confirmed by *T. gondii*-specific PCR and immunohistochemistry.

The protozoan parasite *T. gondii* causes zoonotic toxoplasmosis infections worldwide. The infection is usually asymptomatic in immunocompetent hosts; however, intrauterine transmission is a major concern (4, 5). Also, the infection can reactivate in immunocompromised patients and cause severe disease (6, 7). Toxoplasmic encephalitis is the most common parasitic opportunistic central nervous system (CNS) infection in HIV patients; those with a CD4 count less than 100 cells/ μ l are at particularly high risk if not on prophylaxis against toxoplasma (8, 9). The most common clinical presentation includes headache, confusion, fever, seizures, and focal neurological deficits. A definitive diagnosis relies on a compatible clinical presentation, detection of the microorganism in biopsy tissue (either by histological examination [including *T. gondii*-specific staining] or by *T. gondii*-specific PCR), and brain images showing one or more lesions (typically multiple lesions with ring enhancement and edema). Positive serology (IgG positive and, rarely, IgM positive) supports the diagnosis (9–11).

The most likely explanation for the surprising identification of *T. gondii* by 16S rDNA PCR and sequencing is a coincidental hit on the apicoplast genome, which is a remnant of a photosynthetic organ shared by parasites belonging to the phylum Apicomplexa. This phylum includes *T. gondii*, together with other parasites like *Plasmodium*, *Babesia*, and *Cryptosporidium*. The D2LSU sequence analysis performed herein targeted the large-subunit rDNA of *T. gondii*.

Previous studies have detected *T. gondii* by D2LSU sequencing (12); however, none detected the parasite by sequencing 16S rDNA. Figure 2 shows the 16S rRNA plastid gene homology between the sequences of the amplicon and that of a reference strain. Figure 3 shows a similar homology for the partial D2LSU 28S rRNA. The figures were produced using the multiple-sequence alignment program ClustalX (EMBL-EBI, Hinxton, United Kingdom).

T. gondii infection of HIV patients showing CNS-manifestations should be considered alongside other infectious and noninfectious causes (6, 9). In this case, had we known or suspected the patient's HIV status, we would have considered a diagnosis of toxoplasmosis and performed specific investigations. This case underscores the importance of immunological evaluation when patients with severe infections do not improve as expected.

Molecular diagnostic techniques occasionally generate results reflecting sample contamination, which are of no clinical relevance. This case, however, illustrates that such techniques are very useful under unusual or special circumstances. In this case, the method revealed a highly relevant clinical finding, even though it was not designed for this purpose. Although there are many different methods for diagnosing parasite infections, novel primers (such as the broad-spectrum primer sets used for 16S and D2LSU rDNA PCR) may be developed to

FIG 2 Partial 16S rRNA plastid gene homology between the *Toxoplasma gondii* strain (ATCC 40050), the clinical sample (LN795826), and the online reference strain from NCBI (JN181049). The colors represent individual A, T, C, and G nucleotides.

FIG 3 Partial D2LSU 28S rRNA gene homology between the *Toxoplasma gondii* strain (ATCC 40050), the clinical sample (LN795827) and the online reference strain from NCBI (AF076901). The colors represent individual A, T, C, and G nucleotides.

enable parasitological identification. These tests could supplement standard diagnostic methods.

In conclusion, 16S and D2LSU analyses identified *T. gondii* infection as the cause of the cerebral lesion and neurological manifestations in this patient. The diagnosis was confirmed by standard tests for toxoplasmosis. This unusual diagnostic work-up made a significant contribution to the diagnosis of a parasite infection.

Nucleotide sequence accession numbers. The DNA sequences detected herein have been submitted to the European Molecular Biology Laboratory under accession numbers LN795826 (*T. gondii* plastid partial 16S rRNA gene, strain ME49) and LN795827 (*T. gondii* partial 28S rRNA gene, strain D2LSU).

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We have no conflicts of interest to declare.

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