JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3783–3784 Vol. 47, No. 11
0095-1137/09/$12.00 doi:10.1128/JCM.00927-09


# False-Positive PCR Detection of *Tropheryma whipplei* in Cerebrospinal Fluid and Biopsy Samples from a Child with Chronic Lymphocytic Meningitis[▽]

Daniel Goyo,[1] Ana Camacho,[2] Carmen Gómez,[3] Rogelio Simón de las Heras,[2] Joaquín R. Otero,[3] and Fernando Chaves[3]*

*Servicio Neurología,[1] Sección de Neurología Infantil,[2] and Servicio de Microbiología,[3] Hospital Universitario 12 de Octubre, 28041 Madrid, Spain*

Received 12 May 2009/Returned for modification 31 July 2009/Accepted 1 September 2009

**We report the case of a teenager with chronic lymphocytic meningitis for whom *Tropheryma whipplei* 16S rRNA PCR results were positive in two cerebrospinal fluid samples and one duodenal biopsy specimen. PCR targeting another specific sequence of *Tropheryma whipplei* and sequencing of the initially amplified 16S rRNA fragment did not confirm the results.**

## CASE REPORT

A 13-year-old boy went to the emergency room with a persistent headache which lasted for 2 weeks and nausea, vomiting, and dizziness. His past medical history included *Helicobacter pylori* infection (which was treated successfully) and unspecific abdominal upper pain lasting for 2 years. Upon examination, the patient was afebrile, and the neurological exam disclosed only bilateral papilledema and meningeal syndrome. Routine laboratory analysis and a computed tomography scan of the brain were normal, so a lumbar puncture was performed. The opening pressure was 390 mm $H_2O$ with a white blood cell count of 195 cells/ml (98% mononuclear cells), protein level of 93 mg/dl, and glucose level of 55 mg/dl. Samples were sent for Gram and Ziehl-Neelsen staining; viral, bacterial, mycobacterial, and fungal cultures; and PCR testing for herpes simplex virus and enterovirus. All results, as well as those of serologies, were negative for human immunodeficiency virus, cytomegalovirus, Epstein-Barr virus, cysticercosis, toxoplasmosis, *Borrelia burgdorferi*, *Brucella abortus*, and *Mycoplasma pneumoniae*. After 24 hours, the patient remained asymptomatic without specific treatment (just analgesics and antiemetics). Cranial MRI was normal, and rheumatologic testing showed no abnormalities. He was diagnosed with subacute lymphocytic meningitis of unknown origin. Two weeks after being discharged, the headaches and vomiting recurred, so another lumbar puncture was performed, revealing 125 white blood cells/ml (98% mononuclear cells), protein level of 62 mg/dl, and glucose level of 59 mg/dl. The new assessment of cerebrospinal fluid (CSF) samples included cytology, adenosine deaminase activity, cryptococcus antigen, and VDRL and serology tests for human immunodeficiency virus, cytomegalovirus, Epstein-Barr virus, cysticercosis, and toxoplasmosis. These tests were negative once again.

Even though the suspicion for Whipple's disease was very low, PCR for *Tropheryma whipplei* was performed with the two CSF samples. The amplification by PCR was performed in a LightCycler thermocycler (Roche), using the primers Whip1 (5′-AGAGATACGCCCCCCGCAA-3′) and Whip2 (5′-ATT CGCTCCACCTTGCGA-3′), which targeted a 266-bp sequence of the 16S rRNA gene (11). The PCR was performed using the following protocol: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 62°C for 7 s, extension at 72°C for 10 s, and a melting curve. The PCR of the CSF samples yielded an apparently positive result, based on the amplification and melting curve and the agarose gel electrophoresis of the PCR product, in the two CSF samples. In order to confirm the Whipple's disease diagnosis, an intestinal biopsy was performed. Histological examination of duodenal biopsy specimens did not reveal periodic acid-Schiff stain-positive macrophage inclusions. However, the 16S rRNA PCR also gave a positive result.

Due to the conflicting results between the diagnostic methods, and taking into consideration that this patient did not present the clinical features of classic Whipple's disease (5) and that pediatric cases are extremely rare (2), we decided to perform a new PCR targeting two specific sequences repeated seven times in the genome of *Tropheryma whipplei* (4). The results of these two PCRs were negative for the CSF and intestinal samples. Then, in order to clarify the specificity of the 16S rRNA PCR product obtained in our assay, we decided to sequence the amplicons in both directions by using the BigDye chemistry kit (version 3.1; Applied Biosystems). The sequences obtained with the 16S rRNA primers yielded a 285-bp sequence that was 95.4% identical to that of *Homo sapiens* chromosome 15 (GenBank accession number AC090868). Sequence alignments of the 285-bp fragment with the 16S rRNA sequence of the strain TW08/27 did not show homology, with the exception of the primers used. Sequence alignments of the 285-bp fragment with the partial sequence of the *Homo sapiens* chromosome 15 are shown in Fig. 1.

In the following days, the patient did not have any additional neurological manifestations, nor did he show other symptoms suggestive of Whipple's disease. The patient completed a treat-

* Corresponding author. Mailing address: Servicio de Microbiología, Hospital Universitario 12 de Octubre, Avenida de Córdoba sn, Madrid 28041, Spain. Phone: (34) 91-3908239. Fax: (34) 91-5652765. E-mail: fchaves.hdoc@salud.madrid.org.

▽ Published ahead of print on 9 September 2009.


FIG. 1. Sequence alignments of the 285-bp DNA fragment after 16S rRNA PCR of the CSF sample with the partial sequence of the *Homo sapiens* chromosome 15. Underlined and boldface letters indicate the primers used.

ment with corticosteroids, and he progressed satisfactorily. He was discharged with the diagnosis of chronic lymphocytic meningitis of uncertain etiology. After an 8-month follow-up, the patient remained free of symptoms.

---

*Tropheryma whipplei* is responsible for Whipple's disease, a rare, chronic, multisystemic infectious disorder, commonly associated with neurological symptoms, with special incidence in adult white men, though it can affect individuals of any age (1, 3, 5, 6, 10). The diagnosis of Whipple's disease is complicated because clinical symptoms are not specific, and *T. whipplei* is very difficult to culture. In cases of gastrointestinal disease, diagnosis is based on the visualization of periodic acid-Schiff stain-positive microorganisms inside the macrophages. Other approaches involve demonstrating the presence of *T. whipplei* by electron microscopy or by the detection of its DNA, using specific PCRs on biopsied tissue samples. Diagnostic PCR assays targeting various parts of the 16S rRNA gene or other specific targets have become an important diagnostic tool (3–5, 7, 8, 10, 11).

In this clinical case, using a 16S rRNA PCR protocol similar to that previously reported (11) for the detection of *Tropheryma whipplei*, the sequence obtained was a false-positive result. Although the sequences of primers used in the 16S rRNA PCR were not totally homologous to the sequence of chromosome 15 (63.2% for Whip1 and 72.2% for Whip2), an amplicon of similar size to the 16S rRNA *Tropheryma whipplei* sequence was generated under the conditions of our Light Cycler PCR assay. PCR testing different specific target genes and sequencing of the amplicon ruled out Whipple's disease in this clinical case. False-positive results of tests using *T. whipplei* 16S rRNA PCR primers, apparently due to sequence homology with bacteria in the oral cavity, have previously been described (9), reinforcing the need to test the specificity of designed primers. This case demonstrates that PCR products of the presumably appropriate size may be derived from organisms (or DNA fragments) other than *T. whipplei*, so an additional test is recommended to confirm the PCR results, especially if the clinical features are not typical or in the absence of histopathological evidence (9). Therefore, positive PCR results by the use of the 16S rRNA gene should be interpreted cautiously, and sequencing should be the norm.

This allows us to suggest that the 16S rRNA PCR results of unusual and atypical cases of Whipple's disease must be interpreted cautiously. PCR results must always be interpreted taking into account clinical features and histopathological results. Because of its broad spectrum of symptoms, Whipple's disease mimics other chronic inflammatory diseases, and considering that *T. whipplei* DNA has been found in people without Whipple's disease (4), the final diagnosis should be done using a combination of classic and alternative evaluations in a hierarchical scheme as recommended previously (4, 5).

D.G. is a recipient of a grant from Fundación Carolina/BBVA. This work was funded, in part, by Ministerio deSanidad y Consumo, Instituto de Salud Carlos III—FEDER, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008).

## REFERENCES

1. **Bermejo, P., and A. Burgos.** 2006. Enfermedad de Whipple y sistema nervioso central. Med. Clin. (Barc.) **127:**379–385.
2. **Duprez, T. P. J., C. B. G. Grandin, C. Bonnier, C. W. Thauvoy, J. F. Gradisseux, J. L. Dutrieux, and P. Evrad.** 1996. Whipple disease confined to the central nervous system in childhood. Am. J. Neuroradiol. **17:**1589–1591.
3. **Dutly, F., and M. Altwegg.** 2001. Whipple's disease and "*Tropheryma whippelii*." Clin. Microbiol. Rev. **14:**561–583.
4. **Fenollar, F., M. Trani, B. Davoust, B. Salle, M. L. Birg, J. M. Rolain, and D. Raoult.** 2008. Prevalence of asymptomatic *Tropheryma whipplei* carriage among humans and nonhuman primates. J. Infect. Dis. **197:**880–887.
5. **Fenollar, F., X. Puéchal, and D. Raoult.** 2007. Whipple's disease. N. Engl. J. Med. **356:**55–66.
6. **Louis, E. D., T. Lynch, P. Kaufmann, S. Fahn, and J. Odel.** 1996. Diagnostic guidelines in central nervous system Whipple's disease. Ann. Neurol. **40:** 561–568.
7. **Ramzan, N., E. Loftus, L. J. Burgart, M. Rooney, K. P. Batts, R. H. Wiesner, D. N. Fredricks, D. A. Relman, and D. H. Persing.** 1997. Diagnosis and monitoring of Whipple disease by polymerase chain reaction. Ann. Intern. Med. **126:**520–527.
8. **Relman, D. A., T. M. Schmidt, R. P. MacDermott, and S. Falkow.** 1992. Identification of the uncultured bacillus of Whipple's disease. N. Engl. J. Med. **327:**293–301.
9. **Rolain, J. M., F. Fenollar, and D. Raoult.** 2007. False positive PCR detection of *Tropheryma whipplei* in the saliva of healthy people. BMC Microbiol. **7:**48.
10. **Schneider, T., V. Moos, C. Laddenkemper, T. Marth, F. Fenollar, and D. Raoult.** 2008. Whipple's disease: new aspects of pathogenesis and treatment. Lancet Infect. Dis. **8:**179–190.
11. **von Herbay, A., H. J. Ditton, and M. Maiwald.** 1996. Diagnostic application of a polymerase chain reaction assay for the Whipple's disease bacterium to intestinal biopsies. Gastroenterology **110:**1735–1743.