Absence of Neospora caninum DNA in Human Clinical Samples, Spain

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Low antibody titers to Neospora caninum have been reported in humans, but infection has not been confirmed. We used N. caninum–specific PCR to test 600 clinical samples from patients with toxoplasmosis signs but Toxoplasma gondii–negative PCR results. We did not detect N. caninum DNA, demonstrating it is an unlikely opportunistic zoonotic agent.

The coccidian parasite Neospora caninum (Apicomplexa: Sarcocystidae) is a major abortifacient agent in ruminants, especially cattle. It is phylogenetically close to Toxoplasma gondii (1), a parasite of high prevalence in humans, but biologically different. N. caninum parasites have a restricted host range but can infect primates (2,3).

N. caninum infection causes neuromuscular disease in dogs and reproductive disorders in ruminants, causing fetal loss due to vertical transfer of parasites during acute infections or reactivation of chronic infections. Clinical neosporosis in animals resembles the disease outcome of toxoplasmosis (1).
of these findings is uncertain because neither parasite DNA nor viable parasites have been demonstrated in human tissues. Unconfirmed reports of *N. caninum*–specific antibodies in the human population (4,5) prompted us to test specifically for *Neospora* DNA in human clinical specimens and assess its possible role in human illness.

We obtained 600 DNA samples from a collection of anonymized human clinical samples from the National Registry of Biobanks (no. C.0004715) in Spain that were deemed exempt from a second ethics approval. Our criteria for selection included any pregnancy-related disorder affecting women or fetuses, toxoplasmosis-like clinical signs or suspicion of toxoplasmosis, and negative results for *T. gondii*–specific real-time PCR (6) and nested PCR (7) (Table).

We isolated total DNA using a QIAamp DNA Mini Kit (QIAGEN, https://www.qiagen.com) and used a single-tube nested PCR to amplify the *N. caninum* internal transcribed spacer 1 region using external primers NN1–NN2 and internal primers NP1–NP2, as previously described (8,9). We expected a diagnostic 249-bp fragment. In each batch of amplifications, positive PCR controls included genomic DNA of 10, 1, and 0.1 *N. caninum* tachyzoites. Using these PCR methods, we found that the analytical sensitivity was <1 tachyzoite of *Neospora* spp. or *T. gondii*.

We did not detect *N. caninum*–specific DNA in the samples analyzed. Previously, transplacental neosporosis was experimentally demonstrated in rhesus macaques (*Macaca mulatta*) in the United States (2,3). A literature review summarized reports of unconfirmed presence of antibodies against *N. caninum* in patients with neurologic disorders, pregnant women, and healthy people, including blood donors (1). Findings of *N. caninum* IgG in HIV-infected patients from Brazil and France (4,5) are of special interest because of possible association with *T. gondii* infections.

We believe *N. caninum* parasites are an unlikely opportunistic zoonotic agent. Application of direct methods for parasite detection in a greater number of samples from HIV-positive patients should complement unclear serologic findings to fully dispeal suspicion of human neosporosis.

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Dr. Calero-Bernal is a postdoctoral researcher at the Saluvet Research Group of the Complutenum University, Madrid. His major research interests are Apicomplexan parasites of zoonotic interest, epidemiology of foodborne parasites, and molecular pathways in virulence and drug susceptibility in protozoans.

**References**


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