Molecular Diagnosis of Polycystic Echinococcosis Due to *Echinococcus vogeli* in a Paraguayan Immigrant in Argentina

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Polycystic echinococcosis due to *Echinococcus vogeli* is a rare parasitic infection that occurs in rural areas of Central and South America. Only molecular identification performed on formalin-fixed paraffin-embedded liver tissue samples gave an unequivocal diagnosis of this disease in a Paraguayan immigrant in Argentina.

CASE REPORT

A 37-year-old male immigrant from Paraguay presenting with jaundice and abdominal pain in the right hypochondriac region was hospitalized in Cosme Argerich Hospital, Buenos Aires, Argentina. An abdominal computed tomography (CT) scan revealed a large hypodense liver lesion (11 by 7 cm) in segments IV and VIII with multiple cystic vesicles and irregular margins (Fig. 1). No cystic lesions were observed in the abdominal cavity or chest. The biliary obstruction was initially treated with percutaneous biliary drainage; a secondary infection of the lesion followed this treatment. The abscess was drained percutaneously, and a biopsy specimen was taken from its peripheral margin. The biopsy revealed the presence of cyst layers evoking echinococcosis, but there was no evidence of scolices or rostellar hooks. The diagnosis of echinococcosis was confirmed by positive serology: enzyme-linked immunosorbent assay (ELISA) using *Echinococcus granulosus* crude fluid antigen (Bordier Affinity Products, Crissier, Switzerland), immunoelectrophoresis with arc 5 positivity (crude *E. granulosus* cyst fluid antigen), and Western blotting (LD BioProducts, Lyon, France) (Fig. 2). At radiological imaging and analysis of pathological data, alveolar echinococcosis (AE) was first hypothesized, although the patient had never been in the Northern Hemisphere. The patient was discharged with albendazole treatment (800 mg/day). Because of persistent suppurative drainage, atypical surgical liver resection that included segments IV and VIII was performed 7 months later; the biliary catheter was left in place. Histopathology of the operative liver specimen confirmed the hypothesis of AE. There was a polycystic lesion, showing cyst layers surrounded by granulomatous reaction with histocytes (Fig. 3). Because of the postoperative biliary fistula, surgeons performed a right hepatectomy. The patient was discharged with albendazole treatment and his follow-up stopped. He died 2 years later from pneumonia (he also had AIDS coinfection).

Parasite DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) operative liver tissue samples (five cuts; tissue area, 2 cm²; thickness, 5 μm), using NucleoSpin tissue (Macherey-Nagel, Düren, Germany) with a xylene pretreatment step following the manufacturer’s recommendations. Two PCRs were conducted for amplification of *Echinococcus* mitochondrial DNA (mtDNA). One PCR amplified a 155-bp DNA fragment cytochrome b (*cob*) using previously described forward primer Cob-f (5′-GTCAGATGTCTTATGGGCTGC-3′) (1) and a new reverse primer Cob-r2 (5′-AAACCCAAACAAATATGAACC-3′), and one PCR amplified a 276-bp target on mitochondrial cytochrome c oxidase subunit 1 (*cox1*) using EgCox1-876F (5′-GTTTACTGT TGGGTGTAGTG-3′) and previously published EgCOI-F (5′-TA ACGACATAACATAATGAAAATG-3′) (2). Amplicons were sequenced using a BigDye terminator v3.1 kit (Applied Biosystems, Foster City, CA, USA) with the same primers used in the PCR step using the ABI Prism 3130 DNA analyzer (Applied Biosystems). The sequences were compared to those available in the GenBank database using BLAST software (http://www.ncbi.nlm.nih.gov/blast). Both sequences showed 100% homology with the *E. vogeli* complete mitochondrial genome (GenBank accession number AB208546) (3). The identities of the two sequences with other *Echinococcus* species were much lower: 88% for *cob* and 92% for *cox1* with *Echinococcus oligarthrus*, 89% and 91% with *Echinococcus multilocularis*, 91% and 93% with species belonging to *E. granulosus* sensu lato group, respectively. Thus, a retrospective diagnosis of polycystic echinococcosis (PE) due to *E. vogeli* was made.

*Echinococcus vogeli* is a neotropical parasite which causes polycystic echinococcosis (PE) in humans in the rural areas of Central America and northern South America, mainly in Brazil. *E. vogeli* is found in humid tropical forests and has primarily a sylvatic life cycle involving bush dogs (*Speothos venaticus*) and domestic dogs (*Canis familiaris*) as definitive hosts, and pacas (*Cuniculus pacu*) and agoutis (*Dasyprocta aguti*) as natural intermediate hosts (4). Cystic echinococcosis (CE), due to *E. granulosus*, is highly endemic in livestock-raising countries of the southern part of South America, especially Argentina (5). A total of 172 cases of PE were reported up to 2007, but the actual prevalence of human PE has probably been underestimated (4, 6). Formal identification of
parasite species based on morphology and size of parasite hooks was available in only one-third of cases, mainly *E. vogeli*; cases of echinococcosis caused by species closely related to *E. vogeli*, *Echinococcus oligarthrus* and *E. granulosus*, were rare (4).

A century ago in Argentina, Marcelo Viñas reported several cases of multicystic parasitic hepatic disease that he called “alveolar echinococcus” (7,8). In these cases, as in the present case, the histopathology was very similar to that of AE, but no *Echinococcus* hooks were observed. However, *E. multilocularis*, which causes AE, is now known to be restricted to temperate, holoarctic regions (9). Thus, Viñas’ cases were later categorized as PE by others, although proof of *E. vogeli* infection was never formally established (4,9). To our knowledge, our case is the first unequivocally proven case of PE due to *E. vogeli* diagnosed in Argentina.

Our patient was originally from Paraguay, but PE due to *E. vogeli* has never been reported in Paraguay, even though parasite intermediate hosts (pacas and agouti) are present in this country, and also in northern areas of Argentina (4). However, we could not determine the exact geographical origin of the patient’s contamination, because he lived in rural areas of both countries. In countries such as Argentina where CE is highly endemic (10), clinicians need to be aware that the diagnosis of PE is possible when multicystic lesions are observed, especially in immigrants originating from the rural areas of Central America or northern South America.

At present, there are no clear guidelines for the management of PE patients. *E. vogeli* is thought to be the most pathogenic species of the genus *Echinococcus*, associated with poor outcome (29% mortality rate) (4). As higher survival rates have been observed in patients treated surgically in combination with chemotherapy, a better management of PE would probably be to duplicate that of AE. It would include, therefore, radical surgery, if possible, as soon as possible, followed by long-term albendazole therapy (4, 11).

Given the higher mortality risk of PE, diagnosis of the exact causative *Echinococcus* species is required when multicystic parasitic lesions are observed. No specific serological tools are available for *E. vogeli*. Patient serologies often show high reactivity against *E. granulosus* or *E. multilocularis* antigens, and without a specific species pattern, the diagnosis will only be that of echinococcosis. This lack of specificity could also lead to PE being misdiagnosed as CE or AE (12, 13).

Histopathology remains the “gold standard” for the diagnosis of PE, but the definitive diagnosis of *E. vogeli* infection cannot be made when hooks are not present, as was the case for our patient. Recently, molecular tools were used on a fresh operative specimen...
to confirm diagnosis of PE due to *E. vogeli* in a patient from French Guiana, and the results were in agreement with hook morphology (14). In our case, the FFPE liver tissue samples were the only available specimen for further molecular study. Amplification failed using previously published pan-*Echinococcus* PCR targeting mtDNA genes (*cox1* and *cob* data not shown) (1, 2, 4, 15) or *E. vogeli-*specific PCR (14). As these techniques require amplification of long DNA fragments (>400 bp), we designed new primers, which target DNA polymorphic sequences (<300 bp), i.e., *cox1* and *cob*. Thus, we were able to identify *E. vogeli* in this sample. This underlines the difficulty of DNA extraction from FFPE (degradation of DNA and low yield of extraction of long DNA fragments) and the need for shorter PCR targets for identification of infectious agents using PCR and sequencing in FFPE tissue (16–18). An alternative quantitative PCR (qPCR) approach that targets short mitochondrial DNA fragments could also be of interest on FFPE DNA extract, either by combining species-specific targets or by defining genus-specific system, followed by melting curve analysis for species diagnosis (19, 20). However, given the genetic variability of this parasite, the design of the specific primers and probes must be based on sequence data from a wide panel of *E. vogeli* samples (21).

In this case report, we report the first proven case of PE from Paraguay, diagnosed by PCR followed by sequencing on FFPE tissue. This case underlines the difficulty in diagnosing PE in South America, especially in areas where CE is highly endemic. PE must be suspected in any patient coming from an area where *E. vogeli* is endemic who presents with multicystic lesions and a highly positive *Echinococcus* serology. Molecular tools and/or hook sizing must be used to make this differential diagnosis and to optimize patient management.

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**REFERENCES**
