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Primary Cerebral Alveolar Echinococcosis: Mycology to the Rescue

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A case of primary cerebral alveolar echinococcosis with a favorable outcome is reported. A universal fungal PCR enabled this diagnosis, while the initial serological analysis remained noncontributive.

CASE REPORT

This case is concerning a 62-year-old retired man who lived in the region of the Vosges, in France, near the forest. He used to eat fruits and vegetables from his own garden. The patient was diabetic and hypertensive and had undergone surgery 7 months earlier for aortic stenosis with establishment of an aortic mechanical heart valve. He was hospitalized in Nancy University Hospital for balance disorders evolving over 2 months, with ataxia and appearance of a right-side hemiparesis. These symptoms led to our performing a brain magnetic resonance imaging (MRI), which showed a left occipital mass of 3 cm in diameter, calcified and surrounded by a large perilesional edema (Fig. 1A). The first diagnostic orientation was in favor of a glial tumor. Nevertheless, because of the onset of fever, endocarditis was then discussed, since the results of a transesophageal echocardiography showing vegetations of the mechanical valve were compatible with this suspicion. Thus, septic emboli were suspected on the basis of brain images. Concerning the biological analyses, the results of blood cultures and serology to detect *Coxiella burnetii*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Rickettsia*, *Bartonella henselae*, *Bartonella quintana*, *Brucella*, and HIV were negative. The results of analysis performed to detect *Tropheryma whipplei* in blood, saliva, and feces were negative. The results of serology to detect *Echinococcus granulosus* (indirect hemagglutination Fumouze kit titer, 80) and *Echinococcus multilocularis* (enzyme-linked immunosorbent assay [ELISA] *Echinococcus multilocularis* Bordier Affinity Products index, 0.731) were also negative. There was at that moment no eosinophilia (0.010 g/liter). A test antibiotic treatment was started with vancomycin, gentamicin, and rifampin replaced by daptomycin, linezolid, and rifampin because of the onset of renal failure. After 4 weeks of treatment, MRI results showed an increase of contrast in the cerebral mass, leading to a stereotactic brain biopsy in order to clarify the diagnosis. The histopathological examination showed eosinophilic material composed of fragments of a laminated layer, intensely colored by the periodic acid-Schiff stain (Fig. 2) and positive by Grocott's methenamine silver stain, without a germinative layer. The results of bacteriological analyses performed by direct examination, standard culture, universal bacterial PCR (16S), specific culture, and PCR of *Mycobacterium* sp. were negative. The fungal culture result was negative, but universal fungal PCR using primers ITS1 (5'-TCCG TAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTG ATATGC-3') resulted in an amplification that revealed, after se-

quencing, the presence of *Echinococcus* sp. DNA. These data were confirmed by a specific homemade *Echinococcus* PCR targeting the NADH dehydrogenase 1 gene (ND1F [5'-GCGTCTCGAAGA TGGGTAGT-3'] and ND1R [5'-CGAACACGTGGTAATGTCC C-3']) and the COX1 gene (COX1F [5'-TTGAATTTGCCACGT TTGAATGC-3'] and COX1R [5'-GAACCTAACGACATAACAT AATGA-3']). Given the originality of this case, tandemly repeated multilocus microsatellite EmsB was used to genotype the EmsB profile for this *Echinococcus multilocularis* strain which was clustering closely with other European isolates of fox origin (G23) (1). At 6 weeks after the first serological test for echinococcosis, a second analysis was made and anti-*Echinococcus multilocularis* antibodies (index, 2.807) were found whose specificity was confirmed by Western blot analysis (LDBio Diagnostics *Echinococcus* Western Blot IgG; bands of 7, 16 to 18, and 26 to 28 kDa) (Fig. 3). Computed tomography (CT) chest, abdominal, and pelvic positron emission tomography scans confirmed the single cerebral localization of the disease. A long-term treatment with albendazole administered at 400 mg twice daily was thus started that has been, until now, well tolerated, allowing an improvement of neurological symptoms after 9 months, with only slight right hemiparesis sequelae (Fig. 1B).

Alveolar echinococcosis (AE) caused by the fox tapeworm metacystode *Echinococcus multilocularis* is observed only in the Northern Hemisphere, and especially in central Europe, Russia/Siberia, Central Asia, western China, the northern region of Japan, and Alaska. Humans become infected through contact with eggs (oncospheres) in the feces of the definitive hosts, most often foxes or dogs, but also wolves, by handling animals or by ingesting contaminated crude vegetables (2). In the present case, these risk factors were exacerbated because the patient lived in a French region with a high prevalence of AE (3).

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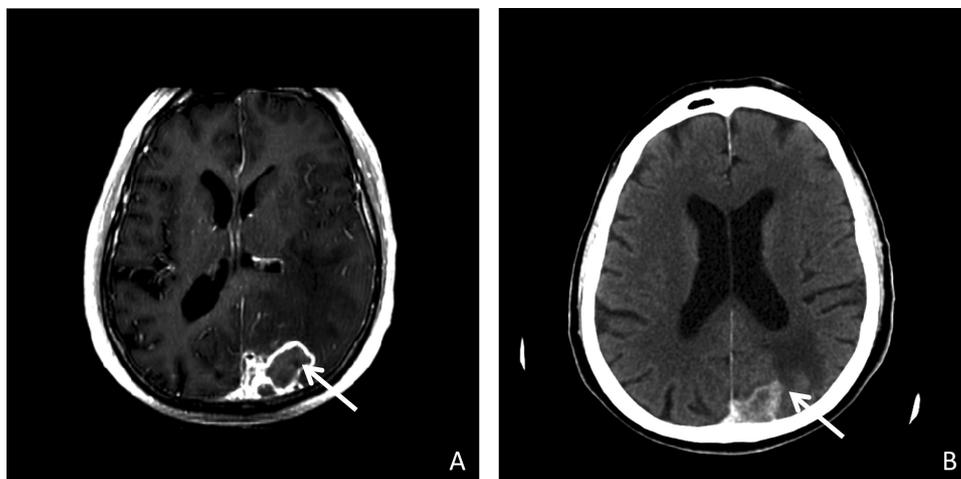


FIG 1 Radiological documentation. (A) Brain MRI at the time of diagnosis. (B) Brain CT scan after 9 months of albendazole treatment.

Occurrences of cerebral *Echinococcus multilocularis* disease are rare, accounting for only 1% of cases, and the disease is generally considered to be fatal. Here, the clinical features were not specific, unlike those usually described. Increased intracranial pressure, epilepsy, neurological disturbances such as dysarthria and hemiparesis, skull deformity, and cranial nerve palsies have been reported (4). Mostly, cerebral metastases are associated with hepatic lesions. Primary alveolar echinococcosis in brain, as in our patient, is exceptional; only 4 case reports are documented in the literature (5, 6).

An EmsB profile from this clinical strain has been observed in Austria and Slovakia (1), but it has never been found in France (G. Umhang, personal communication). According to the literature, primary cerebral AE is not actually known to be associated with specific strains. Moreover, the genetic diversity of clinical strains isolated from AE, with any site considered, has not been explored, in contrast to the results seen with the *Echinococcus granulosus* G6 genotype, which has an affinity for brain of humans (7).

Recently, an approach has been recommended for the immu-

nodagnosis of human AE. Primary antibody tests must include both indirect hemagglutination and at least one ELISA method, performed either with Em2 plus antigen or with recombinant Em18 (recEm18) antigen, because these 3 tests have been proven to have good sensitivity and to yield complementary results. Secondary tests are needed for assessment of the first results by the use of immunoblot analysis. Because of its high specificity, recEm18 immunoblot analysis is particularly recommended in foci in which alveolar and cystic echinococcosis are sympatrically endemic (as in China for example) to fine-tune differential diagnosis. On the other hand, in foci in which AE alone is endemic (as in Europe), the use of LDBio immunoblot analysis (with *Echinococcus multilocularis* crude antigen) should still be recommended because of its excellent sensitivity (8, 9). In our case, immunodiagnosis results were negative when the symptoms began but became positive a few weeks later. This phenomenon may be explained by the unique cerebral localization or by the precocity of the disease.

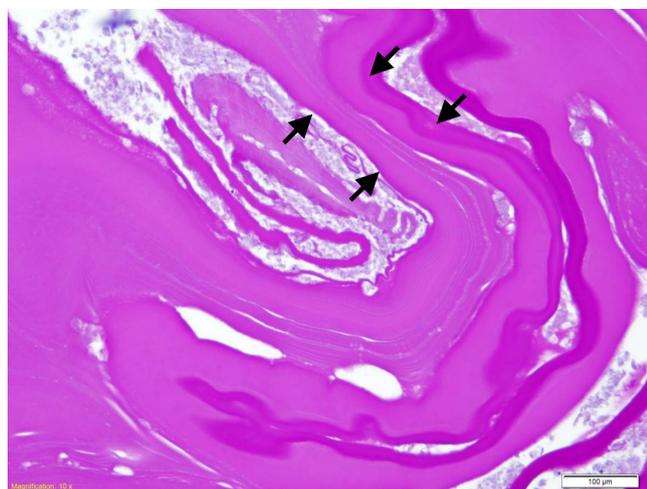


FIG 2 Histopathological examination (magnification, $\times 100$) of brain biopsy sample: the laminated layer (arrows), positive by periodic acid-Schiff staining, without the germinative layer is shown.

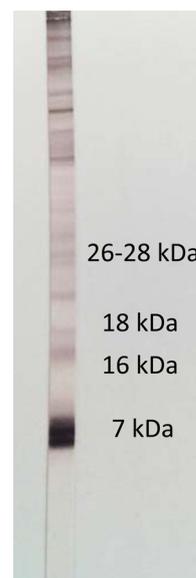


FIG 3 Band profile obtained by Western blot analysis.

Indeed, in an animal (rat) model of cerebral alveolar echinococcosis, the immunoblot results were positive and, more specifically, Em16 and Em18 bands were detected between 11 and 13 weeks after injection of *Echinococcus multilocularis* (10).

Serological misdiagnosis may be due to low *Echinococcus multilocularis*-specific antibody titers and to unusual alveolar echinococcosis localization. In these cases, alternative diagnostic techniques such as PCR or histological examination must be considered (11). In the present case, the diagnosis was made fortuitously by the use of a universal fungal PCR, which was initially used in order to exclude the hypothesis of a fungal infection. These primers (ITS1 and ITS4) target eukaryotic conserved domains of ribosomal genes such as occur in fungi but also in parasites, as in the present case, or in *Toxoplasma gondii* (personal data). In the literature, ITS1 and ITS2 loci have already been used for molecular studies of *Echinococcus* sp. (12, 13). Recently, a few specific PCR protocols for the detection and identification of *Echinococcus* species have been described (11, 14, 15). However, highlighting parasites by using a universal fungal PCR may be helpful when the etiology is unknown by clinicians.

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We declare that we have no conflicts of interest.

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