PRIMARY CEREBRAL ALVEOLAR ECHINOCOCCOSIS: MYCOLOGY TO THE RESCUE

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KEYWORDS

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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 non contributive.
This case is concerning a 62 year old retired man, who is living 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, hypertensive and had undergone surgery seven months earlier for aortic stenosis with establishment of an aortic mechanical heart valve. He was hospitalized in our university hospital for balance disorders evolving over 2 months with ataxia and appearance of a right side hemiparesis. These symptoms lead to the realization of a brain MRI showing a left occipital mass of 3 cm in diameter, calcified and surrounded by a large peri-lesional edema (figure 1A). The first diagnostic orientation was in favor of a glial tumor. Nevertheless, because of the onset of fever, an endocarditis was then discussed all the more that the results of a transesophageal echocardiography were compatible with this suspicion showing vegetations of the mechanical valve. Thus, brain images were suspected of septic emboli. Concerning the biological analyses, blood cultures and serology of *Coxiella burnetii*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Rickettsia*, *Bartonella henselae*, *Bartonella quintana*, *Brucella* and HIV were negative. Research of *Tropheryma whipplei* was negative in blood, saliva and feces. Serology of *Echinococcus granulosus* (IHA Fumouze kit titre: 80) and *Echinococcus multilocularis* (ELISA Echinococcus multilocularis Bordier Affinity Products index: 0.731) were also negative. There was at this moment no eosinophilia (0.010 G/L). A test antibiotic treatment was started with vancomycin, gentamicin and rifampicin replaced by daptomycin, linezolid and rifampicin because of the onset of renal failure. After 4 weeks of treatment, MRI showed an increase of the contrast in the cerebral mass, leading to a stereotactic brain biopsy in order to clarify the diagnosis. The histopathological examination showed an eosinophilic material, composed of fragments of a laminated layer, intensely colored by the periodic acid schiff stain (figure 2) and positive with...
Grocott’ methenamine silver stain, without germinative layer. Bacteriological analyses were negative: direct examination, standard culture, universal bacterial PCR (16S), culture and PCR of *Mycobacterium* sp.. Fungal culture was negative but universal fungal PCR using ITS1-ITS4 primers (ITS1 5’-TCCGTAGGTGAACCTGCGG-3’ and ITS4 5’-TCCTCCGCTTATTGATATGC-3’) resulted in an amplification that revealed, after sequencing, the presence of *Echinococcus* sp. DNA. These data were confirmed by a specific homemade *Echinococcus* PCR targeting the NADH deshydrogenase 1 gene (ND1F 5’-GCGTCTCGAAGATGGGTAGT-3’ and ND1R 5’-CGAACACGTGGTAATGTCGC-3’) and the COX1 gene (COX1F 5’-TTGAATTTGCCACGTTTGAATGC-3’ and COX1R 5’-GAACCTAAGACATAACATAATGA-3’). Given the originality of this case, the 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) [10]. 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 (LDBio Diagnostics Echinococcus Western Blot IgG, bands of 7, 16-18 and 26-28 kDa) (figure 3). CT chest, abdominal and pelvic TEP scan confirmed the single cerebral localization of the disease. A long-term treatment with albendazole 400 mg twice daily was thus started, that is, up to now, well tolerated, allowing an improvement of neurological symptoms with after nine months, only a slight right hemiparesis sequelae (figure 1B).
Alveolar Echinococcosis (AE) caused by the « fox tapeworn » metacestode *Echinococcus multilocularis* is only observed in the northern hemisphere, and especially in central Europe, Russia/Siberia, Central Asia, Western China, north 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 [3]. In the present case, the patient presented these risk factors even more he lived in a French high prevalence region of AE [6].

Occurrence of cerebral *Echinococcus multilocularis* disease is rare, accounting for only 1% of cases and is generally considered to be fatal. Here, the clinical features were not specific, as usually described. Increased intracranial pressure, epilepsy, neurological disturbances such as dysarthria and hemiparesis, skull deformity and cranial nerve palsies have been reported [1]. 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 [9; 11].

EmsB profile from this clinical strain has been observed in Austria and Slovakia [10], but it has never been found in France [Umhang, personnal communication]. According to the literature, primary cerebral AE is not actually known to be associated to specific strains. Moreover, the genetic diversity of clinical strains isolated from AE, any site considered, has not been explored, contrary to *Echinococcus granulosus* G6 genotype which has an affinity for humans’ brain [12].

Recently, an approach has been recommended for the immunodiagnosis of human AE. Primary antibody tests must include both IHA and at least one ELISA method, performed either with Em2 plus antigen or recEm18 antigen, because these 3 tests are proved to have a good sensitivity and yield to complementary results. Secondary tests are needed for assessment of the first results, as immunoblot. Because of its high specificity, recEm18
immunoblot is particularly recommended in foci, for which alveolar and cystic echinococcosis are sympatrically endemic (as in China for example) to fine-tune differential diagnosis. On the other hand, in foci for which AE is only endemic (as in Europe), the use of LDBio immunoblot (with *Echinococcus multilocularis* crude antigen) should still be recommended because of its excellent sensitivity [4; 5]. In our case, immunodiagnosis was 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. Indeed, in an animal model of cerebral alveolar echinococcosis (rats), the immunoblot and more specially Em16 and Em18 bands were detected between 11 and 13 weeks after injection of *Echinococcus multilocularis* [2].

Serological misdiagnosis may be due to low *Echinococcus multilocularis* specific antibody titers and to unusual alveolar echinococcosis localization. In these cases, alternative diagnostic techniques must be considered as PCR or histological examination [8]. In the present case, the diagnosis was made incidentally by 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, as in fungi but also in parasites as in the present case or *Toxoplasma gondii* (personal data). In the literature, ITS1 and ITS2 loci have already been used for molecular studies of *Echinococcus* sp. [7; 15]. Recently, a few specific PCR protocols have been described for the detection and the identification of *Echinococcus* species [8; 13; 14]. However, highlighting parasites by using a universal fungal PCR may be helpful when the etiology is unknown by clinicians.

Conflict of Interest
No conflict of interest


FIGURES

Figure 1: Radiological documentation.

A. Brain MRI at the diagnosis. B. Brain CT-scan after 9 months of albendazole
Figure 2: Histopathological examination (x100) of brain biopsy: laminated layer (arrows), positive with periodic acid shiff staining, without germinative layer.
Figure 3: Band profile obtained by Western Blot
Figure 2