Short communication

Anatomo-clinical and molecular description of liver neotropical echinococcosis caused by *Echinococcus oligarthrus* in human host

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A B S T R A C T

Since humans rarely play the role as *Echinococcus oligarthrus* host, there is lack of knowledge about the complex infectious process. Only three cases have been reported to occur in humans in the neotropics until now. We present the anatomo-clinical and molecular findings describing a new case of infection by *E. oligarthrus* in a man. The muscular or subcutaneous tissues tropism described for this species in the previously reported cases was not present, but a liver tropism was observed. Additionally, the larval stage rostellar hooks morphometry differed from *E. oligarthrus* in the other human cases.

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1. Introduction

Four *Echinococcus* species are known to infect the human host during the larval stage: *Echinococcus granulosus* (Batsch, 1786); *Echinococcus multilocularis* (Leuckart, 1863); *Echinococcus oligarthrus* (Diesing, 1863); and *Echinococcus vogeli* (Rausch and Bernstein, 1972). The latter two are mainly associated with the neotropical echinococcosis in wild areas of Central and South America (D’Alessandro, 1997; Soares et al., 2004). The affected organs vary among the different intermediate host species and also according to the *Echinococcus* species or strain (Thompson and Lymbery, 1988). *E. oligarthrus* is found to infect mainly agouti (*Dasyprocta sp.*) in neotropics and other intermediate hosts (wild rodents) are less frequent. To date, there have been no reports of human echinococcosis caused by *E. oligarthrus* in the Brazilian Amazon. We describe for the first time a case of liver infection by *E. oligarthrus* in a man and present anatomo-clinical and molecular findings.

2. Materials and methods

2.1. Patient

A 62-year-old man presented to a public hospital in the city of Belém (Pará State, Brazil) on 2 February 2011. He showed abdominal bloating and a palpable mass in the right hipocondry. The patient had a longstanding practice of hunting in the Brazilian Western Amazon Forest (0°46′8″S, 47°10′26″W). On 6 February 2011, abdominal computed tomography (CT) scan revealed a hypodense mass in the left hepatic lobe (Fig. 1A). Cranial CT scan and chest radiography were normal. A serologic test (VIRCELL®) was negative for *E. granulosus* IgG antibodies. Despite the negative result, polycystic echinococcosis was a considered hypothesis based on the imaging data and the patient life history. The patient was then submitted to lesion excision. Albendazole was administered immediately after surgery. Five month post-surgery the patient had good health condition. The adopted proceedings received ethical approval from the Instituto Evandro Chagas Ethics Committee.

2.2. Laboratorial cystic material procedures

Fresh (non-fixed) tissues from the patient lesion were obtained by surgery and submitted to parasitological analysis...
and molecular characterization. There were carried out meticulous measurements of 42 large rostellar hooks with a Zeiss Axiophot® microscope using 100× oil-immersion lenses. Tissues were fixed in 10% neutral formalin for histopathology. Sections fixed on glass slides were stained with hematoxylin/eosin (HE) and periodic acid Schiff (PAS), and evaluated using light microscopy. DNA from a cyst was extracted using QIAamp DNA Mini Kit (QIAGEN®), following the manufacturer’s instructions. Molecular identification was carried out by amplification and sequencing of a mitochondrial cytochrome C oxidase subunit 1 gene (COX1) fragment of Echinococcus, as previously described (Bowles et al., 1992). PCR products were purified for sequencing using ExoSAP-IT PCR Clean-up Kit (GE Healthcare), following the manufacturer’s instructions. The purified PCR products were sequenced using the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems) and sequencing in an automatic sequencer ABI 3500 DNA Analyzer (Applied Biosystems).

The nucleotide sequence determined in this study was deposited in the Genetic sequence database at the National Center for Biotechnical Information (NCBI) (GenBank ID: JN367278). This sequence was aligned with sequences of different Echinococcus species using BioEdit 7.0.9.0 and the integrated CLUSTAL W program (Hall, 1999). Phylogenetic analyzes were carried out using the Neighbor-Joining algorithm with Kimura-2 parameters implemented in MEGA v4.1 software (Kumar et al., 2007). Bootstrap analysis with 1000 replicates was performed to determine the robustness of the groups in the phylogenetic tree. A nucleotide sequence of Taenia solium (GenBank ID: AB086256) was used as the outgroup.

3. Results

3.1. Parasitoscopic findings

Microscope examination of the cystic liver specimen revealed protoscolecites with multiple large and small rostellar hooks, consistent with the Echinococcus genus. The detailed analysis of 42 large hooks showed the characteristic shape of E. oligarthrus, since the blade length was slightly smaller, thicker and straighter when compared to E. vogelli (Fig. 1C). The hooks length ranged from 38 to 42 μm (mean: 40 μm). The proportional mean lengths of the handle/blade were respectively 37.5 and 62.5% of the hook total length.

3.2. Pathological findings

Throughout surgery it was observed a multicystic-tumoral mass, occupying liver segments II, III and IV, which was excised. Scenes of the surgery and details of the lesion macroscopic aspect in vivo are available on line (Video data).

The formalin-fixed material (11.5 cm × 10.5 cm × 8.0 cm) presented bulky micronodular lesions, occupying almost the whole surgical piece, with a chestnut-whitish coloration and fibro-firm consistency. The sample surface had a microcystic, spongy, and slightly solid appearance, with whitish/greenish mucinous content and necrotic aspect (Figs. 1B and 2A).

Histopathology showed a multicystic/microcystic pattern in areas adjacent to the liver parenchyma and fibrohistiocyctic connective tissue. Sometimes the microcystic lesions revealed a three-layer wall, typical of hydatid disease cysts (Figs. 1D and 2B).
The inner (germinal) layer had nuclei, while the second avascular (laminated) and third (adventitial) layers were rich in host inflammatory cells. In some areas, the microcysts showed internally integrated brood capsules containing protoscolices (Fig. 1E). Occasional E. oligarthrus larval rostellar hooks of typical morphology were visualized (Fig. 1C). Other areas showed broken brood capsules surrounded by calcifications and chronic inflammatory reaction, rich in histiocytes, lymphocytes, plasma cells, and eosinophils. In the cyst-host tissue interface, we observed a layer of fibrohistiocytic reaction with an incidence of giant cells, as well as a pseudocapsule with linfoplasmocytic infiltration, eosinophilic, and fibrous connective tissue (Fig. 1E). The microcysts rarely appear as a slightly spherical structure that enlarges concentrically. On the other hand they are frequently clustered, just separated by complete or incomplete fibrous septae with invasive proliferation taking place by means of extensions of the germinal layer peripherally, constituting areas of small and interconnected chambers (Fig. 2).

3.3. Molecular findings

COX1 gene analysis confirmed E. oligarthrus as the etiologic agent (Fig. 3). The DNA sequence of the parasite infecting this patient grouped closely (91% bootstrap value) with two published sequences of E. oligarthrus isolated in Panama, but not in the same cluster.

4. Discussion

We report the first viscero-hepatotropic echinococcosis case in a man caused by E. oligarthrus. The intermediate hosts for E. oligarthrus are wild rodents including agouti, spiny rat (Proechimys sp.) and paca (Agouti paca) (D’Alessandro et al., 1981). In regard to larval stage tropism and morphology of the rostellar hooks, E. oligarthrus infection in the animal intermediate host reveals the development of cysts in the muscles, subcutaneous tissues of the rostellar hooks, E. oligarthrus infection in the animal intermediate host reveals the development of cysts in the muscles, subcutaneous tissues (Rausch et al., 1984; Sousa and Thatcher, 1969; Zimmerman et al., 2009) or in parenchymatous organs such as lungs, liver, and spleen (Rausch et al., 1984; Rodriguez et al., 2000). In agouti infections, there is a higher frequency of smaller large hooks (mean: 32 μm) when cysts develop in muscles and subcutaneous tissues compared to parenchymatous organs (mean: 38 μm) (Rausch et al., 1984).

In the three previously described human cases, metacestodes were orbital, found behind the eye, or in the heart muscle. The large hook length, 31.5 (Basset et al., 1998) and 32.6 μm (D’Alessandro et al., 1995), most likely represents cyst development in muscles. Inversely to the other reported cases, we observed hepatotropism and the cyst developed as expected for a parenchymatous organ and large hooks ranging the length from 38 to 42 μm were demonstrated. Handle/blade means of the large hooks were, respectively, 37.5 and 62.5% of their total length. It differs to the previously published hook measurements for the E. vogeli and classic E. oligarthrus (D’Alessandro et al., 1995; Rausch et al., 1984), respectively larger and smaller than the hook length we have found.

Although we have found the shape of large rostellar hooks consistent with E. oligarthrus, their length was very similar to that observed in E. vogeli but higher than the rostellar hooks length in the reported human cases caused by E. oligarthrus. E. oligarthrus infecting agouti in Brazil had been described with rostellar hook lengths higher than those observed in E. oligarthrus from northern regions of South America and Panama (Rausch et al., 1984). However, the E. oligarthrus morphometry has been based on specimens from other countries in Central and South America, including Panama and Colombia (Rausch et al., 1978). The hook measurement in our study is similar to rostellar hook measurements reported for Brazilian E. oligarthrus specimens, whose large hooks ranged in length from 35 to 40 μm (mean: 38.5 μm) (D’Alessandro et al., 1995; Rausch et al., 1984).

The abdominal CT scan and pathological analysis of the gross specimen sections showed a micro and multicystic-tumoral structure (Fig. 1A and B). The microcysts are less than 1 cm diameter and the macroscopic appearance is similar to that seen in alveolar echinococcosis (Pawłowski et al., 2001). Sousa and Thatcher (1969) experimentally infected agouti with E. oligarthrus and described the presence of the internal septae in the larval stage. Nevertheless, the larval stage of E. oligarthrus has been considered unicystic in the natural host (D’Alessandro and Rausch, 2008). The term multicystic is applied to the larval stage for this human case in the sense of ‘multiple cysts closely related’, equivalent to ‘multivesicular’ (Pawłowski, 1997). The Fig. 2(A) and (B) highlights microscopically the development of the microcysts that rarely appear as a slightly spherical structure that enlarges concentrically. On the other hand they are frequently clustered, just separated by complete or incomplete fibrous septae with invasive proliferation taking place by means of extensions of the germinal layer peripherally, constituting areas of small and interconnected chambers (Fig. 2). This morphological appearance is similar to the multivesicular lesions of the alveolar echinococcosis due to E. multilocularis (Rausch and D’Alessandro, 1999). Brood capsules and protoscolices were less frequent. Presence of foreign-body giant-cell reaction, fibrous...
tissue, calcifications, and folded laminated layers also had analogous appearance to alveolar echinococcosis. Cystic invasion of the laminated layer is frequently observed in human infections by *E. vogeli* (Rausch et al., 1984; Meneghelli et al., 1992; Soares et al., 2004), sometimes occupying most of the cyst internal area.

The COX1 gene sequence is closely clustered with *E. oligarthrus* isolated in Panama (Bowles et al., 1992; Nakao et al., 2007) and differed from other *Echinococcus* species that also occur in South America: *E. granulosus* and *E. vogeli* (Fig. 3). However, the different clusters may indicate a geographic isolation of the strains. There is no available information concerning to the intermediate host and tissue tropism related to the COX1 gene sequences of *E. oligarthrus* from Panama. Furthermore, there is no information regarding the sequences of *E. oligarthrus* from the other three cases described in humans as well (Basset et al., 1998; D’Alessandro, 1997; D’Alessandro et al., 1995).

Despite the substantial taxonomic data available, it is difficult to effectively allocate many genetically distinct populations within the *Echinococcus* species (Thompson and Lymbery, 1988). There are circumstances where intraspecific dissimilarities can be very important. Thompson and Lymbery (1988) also emphasize that the genetic variation of parasitic organisms would have great medical significance and the molecular characterization could aid in determining variations.

There is no reference regarding genetic differences within neotropical *Echinococcus*. Additionally, neotropical echinococcosis is a disease that is poorly understood and that requires a complex medical evaluation in order to define the appropriate type of intervention (clinical treatment or surgery). Thus, knowing specific organotropism and the characteristics of the lesions is highly relevant for the prevention, diagnosis, and treatment of the disease.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.actatropica.2012.09.004](http://dx.doi.org/10.1016/j.actatropica.2012.09.004).

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