Neotropical echinococcosis: Second report of Echinococcus vogeli natural infection in its main definitive host, the bush dog (Speothos venaticus)

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Abstract

The bush dog (Speothos venaticus) acts as the natural definitive host in the life cycle of Echinococcus vogeli, the causative agent of polycystic hydatid disease, a zoonotic neglected disease in the South America. We report a case of natural infection by Echinococcus vogeli in a bush dog from the Brazilian Amazon, confirmed by the morphological and morphometric examination of adult parasites and their hooks obtained from the small intestine of the canid. Additionally, mitochondrial DNA sequence analysis corroborated these findings. This is the second report of natural infection by E. vogeli in a bush dog.

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Echinococcus vogeli is the main etiological agent of polycystic hydatid disease or echinococcosis, a disease caused by the development of the larval stage of this tapeworm (metacestode) in man [1]. In the life cycle of E. vogeli, the bush dog, Speothos venaticus (Lund, 1842), acts as the definitive host, and the paca, Cuniculus paca (Linnaeus, 1766), is the major intermediate host, whereas humans are considered an accidental intermediate host [2]. In intermediate hosts, E. vogeli infection is characterized by the formation of cystic or tumorous-like polycystic lesions in various organs such as the liver, spleen and lungs [1]. Due to its distribution – northern South America and parts of Central America – this disease is also called neotropical echinococcosis or hydatidosis [1]. Brazil is the country with the highest incidence of neotropical echinococcosis in the world and the Marajó archipelago, more recently, has been characterized as an enzootic and endemic area of polycystic neotropical echinococcosis in the Brazilian Amazon [3].

The bush dog is a small wild canid, native to the rainforests that span the extreme South of Panama, Colombia, Venezuela, Guyana, Brazil, southern Bolivia, Paraguay and Northern Argentina, which has a diet that includes fish, rodents such as paca and agouti, and larger mammals including capybara [4,5]. Despite a wide estimated geographical distribution, this species is considered rare in the wild making its study in the field difficult, and resulting in a poor understanding of its real status distribution and habits [4–6]. A previous record of this canid’s occurrence on the island of Marajó indicates a possible role for this species in the natural cycle of E. vogeli in the region [6].

Despite the bush dog’s participation in the natural cycle of neotropical echinococcosis caused by E. vogeli, its unusual status in the wild [1] and its protection by Brazilian environmental legislation have meant that, until now, only one instance of natural E. vogeli infection in this canid species has been reported [2].

In late 2007, an expedition from the Evandro Chagas Institute, Belém, Pará, Brazil, developed a fieldwork to investigate parasitic liver diseases in wild rodents hosts of the Brazilian Amazon in the municipality of Anajás, situated on Marajó island, Pará (00 ° 48’ 877” S 049 ° 42’ 06” W). These activities were previously authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) [Environmental License N° 006/2007].

Although the original aim of this work was to search for hydatid cysts in natural intermediate hosts of neotropical echinococcosis such as the paca and agouti, an adult specimen of Speothos venaticus, accidentally killed by a native hunter of the region during subsistence hunting, was delivered to the staff for study. Because of the potential importance of this mammal as the definitive host of E. vogeli, the canid was necropsied to collect stool, tissue and intestinal segment
samples, which were initially packaged for transport in liquid nitrogen and subsequently stored at $-70 \, ^\circ \text{C}$ until analyzed. After proper communication through a report to IBAMA, legal permission was obtained to continue the studies for scientific and sanitary purposes using the previously obtained samples. Finally, the coat of this canid specimen was deposited in the Emilio Goeldi Museum of Pará, located in Belém, Pará, Brazil (code: MPEG-41026).

In a suitable biosecurity laboratory environment, canid fecal samples were submitted to coproparasitologic exams that indicated the presence of eggs with chitinous double layer and hexacanth embryo.

![Image](image_url)

**Fig. 1.** Segment of the small intestine of a bush dog with adult specimens of *E. vogeli* adherent to the mucosa (A); photomicrograph of a large hook (B); an egg of *E. vogeli* with hexacanth embryo within (C) and an adult *E. vogeli* specimen (D) obtained from a natural infection in a bush dog.

Table 1
Morphometric measurements (range) of adult specimens of *E. vogeli* obtained from natural infection of *S. venaticus* on Marajo Island, Eastern Brazilian Amazon, 2007.

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>E. vogeli</em> Marajó, Brazil (mm)</th>
<th><em>E. vogeli</em> Esmeraldas, Ecuadora (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of strobila</td>
<td>3.81 – 8.57</td>
<td>3.94 – 5.58</td>
</tr>
<tr>
<td>Length of gravid segment</td>
<td>2.1 – 5.47</td>
<td>2.94 – 4.2</td>
</tr>
<tr>
<td>Width of the gravid segment</td>
<td>0.53 – 0.86</td>
<td>0.285 – 0.33</td>
</tr>
<tr>
<td>Width of the penultimate segment</td>
<td>0.31 – 0.62</td>
<td>0.232 – 0.285</td>
</tr>
<tr>
<td>Rostellum (length × width)</td>
<td>0.13 – 0.18 × 0.18 – 0.23</td>
<td>0.103 – 0.134 × 0.154 – 0.188</td>
</tr>
<tr>
<td>Diameter of suckers</td>
<td>0.15 – 0.19</td>
<td>0.115 – 0.134</td>
</tr>
<tr>
<td>Genital pore position</td>
<td>Unilateral or alternating/slightly posterior to the middle</td>
<td>Unilateral or alternating/slightly posterior to the middle</td>
</tr>
<tr>
<td>Number of segments</td>
<td>2 – 4</td>
<td>3</td>
</tr>
<tr>
<td>Scolex (length × width)</td>
<td>0.60 – 0.95 × 0.39 – 0.63</td>
<td>0.345 – 0.577 × 0.262 – 0.322</td>
</tr>
<tr>
<td>Ratio (anterior: posterior)</td>
<td>1:0.88 × 1:1.27</td>
<td>1:1.09 × 1:3</td>
</tr>
</tbody>
</table>

a Rausch & Bernstein (1972); mm, millimeters.
After opening a segment of the small intestine of the animal, with the assistance of a magnifying glass, small whitish tapeworms were observed adhered to the mucosa of the organ (Fig. 1, panel A).

The taxonomic identification of these helminths was based on the microscopic observation of 11 uncolored specimens of tapeworm (mature and immature) and of 21 large rostellar hooks (Fig. 1, panel B), mounted in histological laminas and submitted to morphological and morphometrical analysis for parasite species classification.

Parasitological assessment of adult worms indicated that these were cestodes with strobila composed by 2–4 segments, all longer than they were wide. Eight parasites showed characteristics of fully developed strobilae (consisting of 3–4 segments) and 3 appeared to be immature (only scolex and 2 segments). The location of a genital pore on the edges of the mature and gravid segment was posterior to the middle in both, and the size ranged from unilateral to alternating in most cases. The morphometric characteristics of the specimens described in Table 1 gave support to the determination that they were cestodes of the species

E. vogeli.

The analysis of the shape and size of the large hooks (n = 21) was important for taxonomic key identification because they demonstrated morphological and morphometrical characteristics compatible with E. vogeli, according to the parameters previously established [7]: the total length ranged from 0.030 to 0.055 mm (0.044 mm average), the handle length ranged from 0.010 to 0.025 mm (0.019 mm average) and the blade length ranged from 0.008 to 0.035 mm (0.025 mm average). The measurement of 15 parasite eggs showed that their length ranged from 0.025 to 0.040 mm (0.0354 mm average), and their width from 0.025 to 0.035 mm (mean 0.0304 mm) (Fig. 1, Panel C).

Adult specimens of E. vogeli obtained in this study were, generally, of a slightly larger size than had previously been described for this species [2] (Fig. 1, Panel D), although experimental studies in dogs have demonstrated that E. vogeli strobila can reach lengths of up to 12 mm [1], which may explain the differences that exist between the morphometric results of this study and the original description of the parasite. The number of adult specimens and rostellar hooks analyzed may also have affected this result because over 90 parasites specimens were analyzed in the original description [2].

DNA from a pool of adult worms was extracted using the QIAamp DNA mini kit (QIAGEN) and the molecular detection was made using a PCR-based amplification of a 446 bp fragment of mitochondrial cytochrome c oxidase subunit 1 gene (cox1) using primers previously described [8]. The purified amplification products were sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) with ABI 3500 DNA sequencer automatic analyzer. With the assistance of Bioedit 7.0.5.3 software, the consensus sequence obtained from the sample was aligned by CLUSTAL W and integrated with 9 other representative nucleotide sequences available in Genbank/NCBI of the genus Echinococcus and one representative of Taenia solium (GenBank Accession no. AB086256), which was used as an outgroup.

The phylogenetic relationships between the sequences (396 bp) were developed using the MEGA program v5.5 by the Neighbor-joining method with the Kimura 2-parameter substitution model. The reliability of the phylogenetic relationships was determined using bootstrap analysis (1000 replicates) and values above 70% were considered significant for the construction of the tree groups (Fig. 2).

The nucleotide sequence obtained in this study (GenBank Accession no. JX315616) claded as clearly distinct from other Echinococcus species and had a close relationship with the sequence obtained from metacestode of the E. vogeli rodent host in Colombia [9]. This molecular characterization was significant because it corroborated the parasitological examination and confirmed that cestodes of the species E. vogeli had parasitized the bush dog.

Additionally, the nucleotide sequence showed high phylogenetic similarity with two other sequences of E. vogeli previously identified in intermediate hosts (paca and human) from the same geographical region of Marajó island [10], demonstrating the effective participation of the bush dog in the epidemiological cycle of neotropical echinoccosis in the Brazilian Amazon.

These results represent the second record of natural infection by E. vogeli in its main definitive host, the bush dog, and this is the first report of this type from the Brazilian Amazon or any location in Brazil.

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