Short Report: Three-Dimensional Reconstruction of *Echinococcus multilocularis* Larval Growth in Human Hepatic Tissue Reveals Complex Growth Patterns

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Abstract. In this study, we present, for the first time, a three-dimensional digital reconstruction of *Echinococcus multilocularis* larval growth in human tissue. Formalin-fixed, paraffin-embedded hepatic tissues from patients with alveolar echinococcosis were serially sectioned, stained, and areas of larval growth of the parasite were microphotographed. Parasitic structures were reconstructed from multiple digital planes, revealing a root-like network of interconnected vesicles and tubules extending into the periphery of lesions.

Alveolar echinococcosis (AE) is an important parasitic zoonosis caused by the larval stage of the fox tapeworm *Echinococcus multilocularis*. Larvae show infiltrative growth in the liver of rodents, the natural intermediate hosts, and accidentally in other mammals including humans. Spread to adjacent organs and metastasis formation may occur. In the natural rodent host, parasitic growth is mainly vesicularly organized. In contrast, human and non-human primate infection is characterized by a histological pattern showing necrosis, severe inflammation, and intensive fibrosis surrounding irregular vesiculotubular structures of the parasite. Invasive parasitic proliferation around the periphery of the lesion appears to be a continuing process and results in a more or less concentric enlargement. Early studies of human AE by Jahn in 1927 showed communicating vesicles of varying diameter in serial sections. Jahn believed in a continuing herniation process even-tually forming a tree of directly attached vesicles. Rausch, who orally infected North American field voles (*Microtus pennsylvanicus*) in 1954, and Vogel, who conducted similar experiments with the European *Microtus arvalis* in 1977, described centrifugally growing, convoluted tubules in histological sections. These tubules enlarged occasionally and formed vesicular structures and branches in these natural intermediate hosts. Detachment of individual vesicles from the tubular structure was also observed. Ultrastructural and histologic studies of subcutaneously infected Mongolian jirds (*Meriones unguiculatus*) by Eckert, Thompson, and Mehlhorn in 1983 similarly demonstrated thin, infiltrating tubules which form blind-ending branches and fungus-like local expansions of variable diameters. In contrast, Lukaschenko described in 1975 a simple binary fission model by which individual daughter vesicles are formed by septation. In human AE the growth phase is characterized by a pronounced fibrosis. Due to this observation, Vogel assumed that the fibrous layers may restrict parasitic growth and that the root- or coral-like tubovescu-lar structures change direction and form convoluted superstructures.

In this report, we present for the first time a three-dimensional reconstruction of *E. multilocularis* larval growth in human liver tissue. Hepatic tissue samples from three patients with advanced-stage AE (stage IIIa and IIIb) were obtained during surgery. All patients had acquired AE in southern Germany and were treated with albendazole 400 mg twice a day. Formalin-fixed, paraffin-embedded hepatic tissue was sectioned and areas of parasitic growth were identified by light microscopy of periodic acid Schiff-stained sections (Figure 1). Centrally and peripherally growing parasitic structures in the respective lesions were serially sectioned with a tissue thickness of 4 μm, resulting in 15–21 section planes per area of interest. All sections were microphotographed using Olympus analySIS 3.2 software, ColorView I camera, and BX50 microscope (Olympus GmbH, Hamburg, Germany) with a magnification ×200. Individual digital microphotographs were stacked, centered, and each layer was saved as an individual file with Adobe Photoshop 7.0 software (Adobe Systems Inc., San Jose, CA). Using MeVisLab software (Fraunhofer MEVIS, Bremen, Germany), the parasitic structures were segmented in each slice with level set methods (Figure 2). The three-dimensional visualization was computed by smooth interpolation of stacked contours.

The computer-generated, three-dimensional reconstruction of centrally located parasitic structures in human AE lesions showed large communicating vesicles with occasional sac-like protuberances (Figure 3A, B). Interestingly, the reconstruction of parasitic growth in this area is similar to what Jahn had pro-

![Figure 1](image-url)
posed for human AE in 1927. In contrast, digital remodeling of peripheral areas of human AE lesions demonstrated a root-like formation of vesicles interconnected by thin tubules with branches extending to the surrounding host tissue (Figure 3C). This growth pattern closely resembles the network of vesiculotubular structures observed by Rausch, Vogel, and others in the experimentally infected rodents. However, the growth pattern and the configuration of the parasitic structures presented herein may be altered due to the administration of albendazole in all patients from which tissue was obtained for this study.

Vogel observed in histological studies with experimentally infected rodents thin extensions of germinal tissue devoid of an external laminated layer arising from the vesicles, which infiltrate the host tissue peripherally in a similar manner as plant roots penetrate soil. These findings were also confirmed ultrastructurally in the Meriones model. Some of the larger extensions produced an outer laminated layer, however. In human AE, only structures with a thick laminated layer were described histologically, but no ultrastructural studies have been published. These thin infiltrating tubules are possibly the basis for metastasis formation, or recurrence of disease after resection of AE lesions. In view of these previous investigations and the reconstruction of the complex growth pattern presented here, the radical resection of parasitic lesions within a safety margin is reasonably the preferred treatment of human AE.

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