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

Dennis Tappe,* Stephan Zidowitz, Philipp Demmer, Peter Kern, Thomas F. E. Barth, and Matthias Frosch

Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany; Fraunhofer MEVIS Institute for Medical Image Computing, Bremen, Germany; Institute of Pathology, University of Würzburg, Würzburg, Germany; Comprehensive Infectious Diseases Center, Division of Infectious Diseases and Clinical Immunology, University Hospital and Medical Center Ulm, Ulm, Germany; Institute of Pathology, University Hospital and Medical Center Ulm, Ulm, Germany

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 vesicotubular 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.¹ 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 eventually forming a tree of directly attached vesicles.² Rausch, who orally infected North American field voles (*Microtus pennsylvanicus*) in 1954,³ and Vogel, who conducted similar experiments with the European *Microtus arvalis* in 1977,^{2,4} 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.² 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.^{5,6} In contrast, Lukaschenko described in 1975 a simple binary fission model by which individual daughter vesicles are formed by septation.² 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 tubovesicular structures change direction and form convoluted superstructures.²

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. **A–B**, Paraffin section through central parts of the parasitic lesion in human liver. Small and large echinococcal vesicles surrounded by the characteristic laminated layer are embedded in necrotic tissue. The three-dimensionally (3D) remodeled regions are highlighted by black rectangles. Arrows mark the viewing direction of the 3D reconstruction depicted in Figure 3A, B. Periodic acid Schiff stain, magnification ×200. **C**, Periphery of parasitic lesion in human liver. The root-like, expanding tubular parasitic structure is surrounded by a dense mesh of fibrotic tissue. The viewing direction of the 3D reconstruction in Figure 3C is from the top of this section. Periodic acid Schiff stain, magnification ×200. This figure appears in color at www.ajtmh.org.

*Address correspondence to Dennis Tappe, Institute of Hygiene and Microbiology, University of Würzburg, Josef-Schneider-Street 2, 97080 Würzburg, Germany. E-mail: dtappe@hygiene.uni-wuerzburg.de

FIGURE 2. Overlay of contours from *E. multilocularis* larvae in histological serial sections. The contours of the vesicular larval parasite were segmented in each slice with level set methods and stacked. **A**, **B**, and **C**, are overlay of contours from Figure 1A–C, respectively. This figure appears in color at www.ajtmh.org.

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.² These findings were also confirmed ultrastructurally in the *Meriones* model.⁶ Some of the larger extensions produced an outer laminated layer, however. In human AE, only structures with a thick laminated layer were described histologically,⁵ but no ultrastructural studies have been published. These thin infiltrating tubules are possibly the basis for metastasis formation,^{2–6} 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.

Received August 10, 2009. Accepted for publication September 15, 2009.

Acknowledgments: The authors would like to thank Mrs. Birgit Rettenmeier, Institute of Pathology, University Hospital and Medical Center Ulm, for excellent technical assistance.

FIGURE 3. Three-dimensional reconstruction of *E. multilocularis* larval growth in human liver. Models were computed by smooth interpolation of stacked contours. **A**, **B**, and **C**, remodeled data from Figure 2A–C, respectively. This figure appears in color at www.ajtmh.org.

Authors' addresses: Dennis Tappe and Matthias Frosch, Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany, E-mails: dtappe@hygiene.uni-wuerzburg.de and mfrosch@hygiene.uni-wuerzburg.de. Stephan Zidowitz, Fraunhofer MEVIS Institute for Medical Image Computing, Bremen, Germany, E-mail: stephan.zidowitz@mevis.fraunhofer.de. Philipp Demmer, Institute of Pathology, University of Würzburg, Würzburg, Germany, E-mail: phdemmer@gmx.de. Peter Kern, Comprehensive Infectious Diseases Center, Division of Infectious Diseases and Clinical Immunology, University Hospital and Medical Center Ulm, Ulm, Germany, E-mail: peter.kern@uniklinik-ulm.de. Thomas F. E. Barth, Institute of Pathology, University Hospital and Medical Center Ulm, Ulm, Germany, E-mail: thomas.barth@uniklinik-ulm.de.

REFERENCES

1. Rausch RL, Wilson JF, Schantz PM, McMahon BJ, 1987. Spontaneous death of *Echinococcus multilocularis*: cases diagnosed serologically (by Em2 ELISA) and clinical significance. *Am J Trop Med Hyg* 36: 576–585.
2. Vogel H, 1978. How does the alveolar echinococcus grow? [in German]. *Tropenmed Parasitol* 29: 1–11.
3. Rausch R, 1954. Studies on the helminth fauna of Alaska. XX. The histogenesis of the alveolar larva of *Echinococcus* species. *J Infect Dis* 94: 178–186.
4. Vogel H, 1977. On *Echinococcus multilocularis* in southern Germany II. Development of the larval stages and histopathological reactions in common vole, *Microtus arvalis* [in German]. *Tropenmed Parasitol* 28: 409–427.
5. Eckert J, Thompson RC, Mehlhorn H, 1983. Proliferation and metastases formation of larval *Echinococcus multilocularis*. I. Animal model, macroscopical and histological findings. *Z Parasitenkd* 69: 737–748.
6. Mehlhorn H, Eckert J, Thompson RC, 1983. Proliferation and metastases formation of larval *Echinococcus multilocularis*. II. Ultrastructural investigations. *Z Parasitenkd* 69: 749–763.