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Authors: Janovsky, Martin, Bacciarini, Luca, Sager, Heinz, Gröne, Andrea, and Gottstein, Bruno

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## Echinococcus multilocularis in a European Beaver from Switzerland

**Martin Janovsky**,<sup>1,3,4</sup> **Luca Bacciarini**,<sup>1</sup> **Heinz Sager**,<sup>2</sup> **Andrea Gröne**,<sup>1</sup> **and Bruno Gottstein**<sup>2</sup> <sup>1</sup>Center for Fish and Wildlife Health, Institute of Animal Pathology, Länggassstrasse 122, CH-3012 Berne, Switzerland; <sup>2</sup>Institute of Parasitology, University of Berne, Länggassstrasse 122, CH-3012 Berne, Switzerland; <sup>3</sup>Present address: Amt der Tiroler Landesregierung, Veterinärdirektion, Wilhelm-Greilstrasse 25, 6020 Innsbruck, Austria; <sup>4</sup>Corresponding author (email: m.janovsky@tirol.gv.at)

ABSTRACT: Infection with the larval stage of the cestode parasite Echinococcus multilocularis was diagnosed in a European beaver (Castor fiber) in central Switzerland. The animal was hit, run over by a car, and died of trauma. It was in normal body condition and no signs of disease were seen. At necropsy, multiple cystic structures up to 1 cm in diameter were found in the liver adjacent to the hilus. Within the parasite vesicles, multiple protoscolices were visible. The species was determined to be Echinococcus multilocularis by upon polymerase chain reaction and direct immunofluorescence with MAbG11-FITC. This is the first report of Echinococcus multilocularis in European beaver.

*Key words:* Case report, *Castor fiber, Echinococcus multilocularis*, European beaver, liver, parasite, Switzerland.

The European beaver (*Castor fiber*) was extirpated in Switzerland at the beginning of the 19th century and reintroduced between 1957 and 1977 (Winter, 1998). The present population includes approximately 350 animals (Anderegg, 1998). A subadult male beaver weighing 10.0 kg was found dead in August 2000 near a main road in the Canton of Aargau (47°34'N, 08°25'E) close to the River Rhine. It was submitted for necropsy to the Center for Fish and Wildlife Health (Berne, Switzerland). The animal died of trauma and was in normal body condition. The capsule of the liver was ruptured. Near the portal hilus multiple white cysts up to 1 cm in diameter were present (Fig. 1). No pathogenic organisms were isolated from liver, lungs, kidney, and spleen by bacteriologic culture. Pieces of heart, liver, lung, kidney, and spleen were fixed in 10% buffered, neutral formalin. After fixation for 24 hr, tissues were embedded in paraffin; sections were cut at 5 µm and stained with hematoxylin and eosin. Specimens from

the liver were also stained with periodic acid-Schiff (PAS; Armed Forces Institute of Pathology, 1968).

The portal area of the liver contained multiple echinococcal cysts characterized by a homogenously PAS-positive bladder wall (laminated layer) adjoining the inner germinal layer with multiple protoscolices protruding from it. These cystic vesicles were surrounded by multiple layers of mature connective tissue, which was infiltrated by moderate numbers of lymphocytes, macrophages, and neutrophils. The connective tissue also exhibited multifocal mineralization. The hepatic parenchyma adjacent to the parasites showed moderate bile duct proliferation and moderate infiltration by lymphocytes and histiocytes, and a few neutrophils.

Specimens of liver including all visible cystic lesions, were submitted to the Institute of Parasitology (Berne, Switzerland). A polymerase chain reaction (PCR) with BG1 and BG2 primers specific for Echinococcus multilocularis was performed as previously described (Gottstein and Mowatt, 1991; Diebold-Berger et al., 1997). DNA isolated from lesions served as template for the PCR. A 2.6 kb pair fragment from the genome of *E. multilocularis* was obtained. Direct immunofluorescence using monoclonal antibody MAbG11-FITC (Deplazes and Gottstein, 1991) was conducted on the lesions. This monoclonal antibody is species-specific in similar cases of human E. multilocularis infections (Diebold-Berger et al., 1997). In the present investigation, immunostaining of the laminated layer was exhibited.

*Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), one of the most lethal helminthic in-



FIGURE 1. Liver from an European beaver from Switzerland. Cysts in the portal area caused by *Echinococcus multilocularis*.

fections of humans (Gottstein et al., 2001). It is endemic in the northern hemisphere. In central Europe, red foxes (Vulpes vulpes) are the major reservoir of E. multilocularis. Foxes from 21 of 26 Cantons in Switzerland were infected at prevalences varying from 2% to 53%, with an overall mean of approximately 30% (Eckert, 1996). The most common intermediate hosts in central Europe are small rodents such as the common vole (Microtus arvalis), water vole (Arvicola terrestris), followed by bank vole (Clethrionomys glareolus), and muskrat (Ondatra zibethicus) (Schmitt et al., 1997). There are numerous reports of "accidental" intermediate hosts. These include medium size rodents such as swamp beavers (Myocastor cyopus) (Worbes et al., 1989). However, E. multilocularis has not been reported in the European or the American beaver (Castor canadensis) (Addison et al., 1987; Romasov, 1992; Nolet et al., 1997). Piechocki (1973) reported Echinococcus granulosus in a European beaver from Russia without further description.

To our knowledge, this is the first documentation of AE in a beaver, thus demonstrating its susceptibility to infection with *E. multilocularis*. Of note is the morphologic peculiarity of the metacestode exhibiting a cyst-like structure not usually seen in hepatic lesions in mice. Misdiagnosis of E. granulosus as the causative agent can be avoided by using hooklet morphology (Wardle and McLeod, 1968), PCR, and immunostaining. In the present case, hooklets were not detectable in the metacestode.

Increased contamination of the Swiss environment with *E. multilocularis* eggs has been anticipated due to the dramatic increase of affected foxes. This has resulted in a high prevalence in some areas in Switzerland (Gottstein et al., 2001) and finding AE in unusual intermediate hosts such as dogs (Haller et al., 1998).

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