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SHORT COMMUNICATION

The brown hare (*Lepus europaeus*) as a novel intermediate host for *Echinococcus multilocularis* in Europe

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Abstract A typical multivesiculated metacestode tissue has been found in the liver of a European brown hare (*Lepus europaeus*) originating from a northern area of Switzerland. In this study, the causative species was identified as *Echinococcus multilocularis* by appropriate histological and molecular analyses and corresponding DNA sequencing. This is the first confirmation of larval *E. multilocularis* from hares in central Europe. The metacestode tissue contained protoscolices, suggesting that the hare may contribute to the transmission of *E. multilocularis* in Switzerland.

Keywords *Echinococcus multilocularis* · Alveolar echinococcosis · Hare · Diagnosis · PCR

Echinococcus multilocularis is a cestode parasite for which foxes (*Vulpes* spp.) serve as the principal definitive host, which intestinally harbors the adult egg-producing tapeworms. Rodents serve as main intermediate hosts and become infected upon per oral ingestion of parasite eggs. Subsequently, an oncosphere is released, which migrates to the liver, and there develops into a metacestode that—upon production of protoscolices—reaches infectivity for definitive hosts within a few weeks to a few months. Conversely to definitive hosts that do not develop clinical signs, intermediate hosts usually develop disease called alveolar echinococcosis (AE). The main intermediate host species for *E. multilocularis* are voles (e.g., *Microtus*, *Arvicola*, and *Myodes* spp.), but some other small mammals are affected as well (Conraths and Deplazes 2015). The range of accidental "intermediate" hosts presenting AE has continuously increased within the past two decades, including, e.g., dogs (Deplazes and Eckert 2001), primates (Rehmann et al. 2005), and beavers (Janovsky et al. 2002). In other susceptible host species like pigs and wild boars, the *E. multilocularis* metacestode dies out and calcifies before reaching fertility (Deplazes et al. 2005), thus, pigs do not contribute to the maintenance of the life cycle of *E. multilocularis*. With the exception of the Tibetan hare *Lepus oiostolus* (Xiao et al. 2004), Leporidae including rabbits and hares have mostly been regarded as unsuitable intermediate hosts for *E. multilocularis* (Ohbayashi et al. 1971). Conversely, fertile larval *Echinococcus granulosus* infections have been demonstrated in the European brown hare in Argentina (Schantz and Lord 1972; Thakur and Eddi 1982), so that this animal species appears to be part of the *E. granulosus* life cycle on the South American continent. Nevertheless, a few older documents already mention the hare to be potentially infected with *E. multilocularis* but without solid etiological proof. These documentations originate from Russia by Bessonov (1998) and from Germany (Kötsche and Gottschalk 1990).

The European brown hare (*Lepus europaeus*) is an important game species in Europe. It originates from the steppe grasslands of Eurasia and exhibits a relatively high intra- and interpopulation genetic diversity of the major histocompatibility complex (MHC) class II DRB locus (Koutsogiannouli et al. 2014). In Switzerland, a study had been carried out to elucidate the importance of different causes of mortality, which could explain the downward trend of the hare populations in this country (Haerer et al. 2001). Infectious diseases led to death in 15 % of the animals, and cases of pasteurellosis, brucellosis, pseudotuberculosis, tularaemia, listeriosis, and toxoplasmosis were diagnosed. AE has so far never been documented upon pathological/necropsy examinations and molecular

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identification of the parasite in dead hares. In the frame of regular hunting, a hare was shot on 24.10.2014 close to the city of Ste-Croix in the Jura region of Switzerland (exact coordinates N 46° 50′ 25″, E 6° 32′ 15″). Due to the unusual presentation of the liver (suspicion of abscess), it was presented for inspection to the gamekeeper. This organ was submitted for further pathological examinations to the Institut Galli-Valerio in Lausanne, Switzerland. The liver was pathohistologically examined upon hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining of tissue sections (4 μm), and multicystic lesions were detected as shown in Fig. 1a and b. The pathologically altered liver tissue consisted of numerous small vesicles with well-developed germinal and PAS-positive thin laminated layers, and most vesicles also contained mature protoscolices and calcareous corpuscules, overall presenting the features of larval *E. multilocularis*. As cystic metacestodes of other *Echinococcus* species may exhibit some superficial similarities especially in unsuitable or aberrant hosts (Vogel 1957), a molecular analysis of this case was undertaken. Genomic DNA was isolated from the formalin-fixed paraffin-embedded hepatic lesion according to Müller et al. (2003). PCR was carried out as previously reported (Diebold Berger et al. 1997). The amplified sequences (GenBank accession no.: KR870967) were completely identical with those published for *E. multilocularis* (Dinkel et al. 1998). These molecular findings confirmed that the hare was infected with larval *E. multilocularis*.

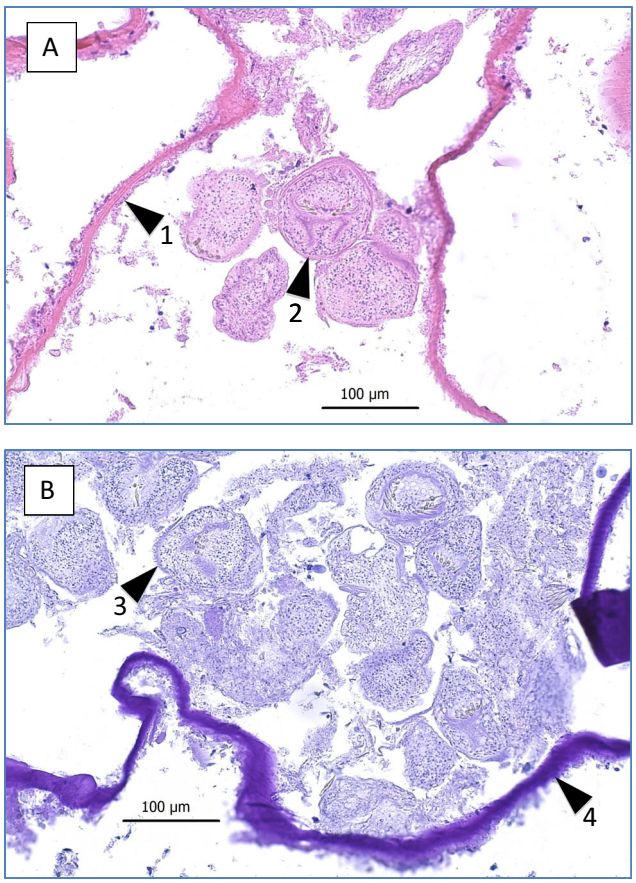

Fig. 1 Histological presentation of the fertile parasitic lesions recovered from the hare's liver. **a** HE-stained section (×200), *arrow no. 1* shows the germinal layer and *no. 2* an intravesicular protoscolex. **b** PAS-stained section (×200), *arrow no. 3* pointing at a protoscolex with a few internal hooks, and *arrow no. 4* shows the PAS-positive laminated layer. *Bar* is 100 μm

Discussion

In Switzerland, the transmission dynamics of *E. multilocularis* depends primarily upon the ecosystem of red foxes (*Vulpes vulpes*) and small mammal intermediate hosts such as *Arvicola terrestris* and *Microtus arvalis* (Hegglin et al. 2015; Guerra et al. 2014). Red foxes are high-prevalence hosts of *E. multilocularis* in Switzerland (Lewis et al. 2014), resulting in a high environmental contamination rate with *E. multilocularis* eggs. As a consequence, larval infections have been increasingly found in "exotic" animal species, for example in beavers, pigs, dogs, and zoo primates (Scharf et al. 2004; Rehmann et al. 2005; Deplazes and Eckert 2001; Janovsky et al. 2002). Most of these accidentally infected host animals cannot directly contribute to the maintenance of the life cycle, as either the metacestodes do not maturate to fertility such as found in pigs, or the accidental hosts do not take part in the dietary spectrum of foxes.

To our knowledge, this is the first report of *E. multilocularis* in the brown hare in central Europe. Our histological observations showed that the infected hare possessed morphologically fully developed and mature protoscolices, suggesting that this animal species could act as a competent intermediate host and thus contribute to the transmission of *E. multilocularis*. Confirmation of this could be achieved after dietary analysis of foxes, such as done to assess the southern European border of *E. multilocularis* (Guerra et al. 2014). The importance of hares as prey species for the red fox in central Europe has already been documented in multiple studies (Knauer et al. 2010; Zellweger-Fischer et al. 2011; Schmidt et al. 2004). Now, the challenge will be to regularly assess any liver lesions detected in hares, as to determine the prevalence of *E. multilocularis* infection in this wildlife animal species. We do not know if in previous times, *E. multilocularis*-induced liver lesions were seen, but were either not histologically investigated, or not at all investigated. Finally, it may be noted that in hares, within a differential diagnosis of macroscopically visible hepatic disorders, other parasites may be the cause of liver lesions, such as *Eimeria stiedae* and *Dicrocoelium dendriticum*, both affecting the biliary system of the liver, and *Fasciola hepatica*, affecting both liver parenchyma and the bile ducts.

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