Parasitology Research

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

--Manuscript Draft--

Manuscript Number:	
Full Title:	The brown hare (Lepus europaeus) as a novel intermediate host for Echinococcus multilocularis in Europe.
Article Type:	Short Communication
Funding Information:	Swiss National Science Foundation (31003A_141039/1) — Prof. Bruno Gottstein
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.
Corresponding Author:	Bruno Gottstein University of Berne Bern, Switzerland
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Berne
Corresponding Author's Secondary Institution:	
First Author:	Valérie Chaignat
First Author Secondary Information:	
Order of Authors:	Valérie Chaignat
	Patrick Boujon
	Caroline F Frey
	Brigitte Hentrich
	Norbert Müller
	Bruno Gottstein
Order of Authors Secondary Information:	
Author Comments:	
Suggested Reviewers:	Maria del Mar Siles Lucas mmar.siles@irnasa.csic.es Expert in echinococcosis
	Franck Boue Franck.BOUE@anses.fr Expert in animal alveolar echinococcosis
	Gerald Umhang Gerald.UMHANG@anses.fr expert in animal echinococcosis
	Thomas Romig thomas.romig@uni-hohenheim.de Expert in animal alveolar echinococcosis

Short Communication

Valérie Chaignat • Patrick Boujon • Caroline F. Frey • Brigitte Hentrich • Norbert Müller • Bruno Gottstein

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

Valérie Chaignat • Patrick Boujon,

Institut Galli-Valerio, Service de la consommation et des affaires vétérinaires, Lausanne, Switzerland

Caroline F. Frey • Brigitte Hentrich • Norbert Müller • Bruno Gottstein (✉)

Institut of Parasitology, Vetsuisse Faculty
University of Bern, Bern, Switzerland
e-mail: bruno.gottstein@vetsuisse.unibe.ch

Key words *Echinococcus multilocularis* • alveolar echinococcosis • hare • diagnosis • PCR

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.

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 peroral 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 *E. 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 inter-population genetic diversity of the 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 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 N46°50'25'', E6°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 patho-histologically examined upon HE and PAS-staining of tissue sections (4 µm), and multicystic lesions were detected as shown in Fig. 1A and 1B. 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 (GeneBank accession no.: submission pending) 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*.

(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 stiedai* and *Dicrocoelium dendriticum*, both affecting the biliary system of the liver, and *Fasciola hepatica*, affecting both liver parenchym and the bile ducts.

Acknowledgements The authors thank Mr Alain Seletto, gamekeeper circ. IV and Mr Daniel Gaille, hunter, for submiting the liver of the hare. The work has been supported by the Swiss National Science Foundation (research grant no. 31003A_141039/1).

References

Bessonov AS (1998) *Echinococcus multilocularis* infection in Russia and neighbouring countries. Helminthologia 35:73-78.

Conraths F, Deplazes P (2015) *Echinococcus multilocularis*: epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet Parasitol. (in press).

Deplazes P, Eckert J (2001) Veterinary aspects of alveolar echinococcosis--a zoonosis of public health significance. Vet Parasitol. 98:65-87.

Deplazes P, Grimm F, Sydler T, Tanner I, Kapel CM (2005) Experimental alveolar echinococcosis in pigs, lesion development and serological follow up. Vet Parasitol. 130:213-222.

Diebold Berger S, Khan H, Gottstein B, Puget E, Frossard JL, Remadi S (1997) Cytologic diagnosis of isolated pancreatic alveolar hydatid disease with immunologic and PCR analyses - A case report. Acta Cytologica 41:1381-1386.

Dinkel A, von Nickisch-Rosenegk M, Bilger B, Merli M, Lucius R, Romig T (1998) Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. J Clin Microbiol. 36:1871-1876.

Guerra D, Hegglin D, Bacciarini L, Schnyder M, Deplazes P (2014) Stability of the southern European border of *Echinococcus multilocularis* in the Alps: evidence that *Microtus arvalis* is a limiting factor. Parasitology 16:1-10.

Haerer G, Nicolet J, Bacciarini L, Gottstein B, Giacometti M (2001) Causes of death, zoonoses, and reproduction in the European brown hare in Switzerland. Schweiz Arch Tierheilkd. , 143(4):193-201

Hegglin D, Bontadina F, Deplazes P (2015) Human-wildlife interactions and zoonotic transmission of *Echinococcus multilocularis.* Trends Parasitol. pii: S1471-4922(14)00207-4. doi: 10.1016/j.pt.2014.12.004. [Epub ahead of print].

Janovsky M, Bacciarini L, Sager H, Gröne A, Gottstein (2002) *Echinococcus multilocularis* in a European beaver from Switzerland. J Wildl Dis. 38(3):618-620.

Knauer F, Küchenhoff H, Pilz S (2010) A statistical analysis of the relationship between red fox *Vulpes vulpes* and its prey species (grey partridge *Perdix perdix*, brown hare *Lepus europaeus* and rabbit *Oryctolagus cuniculus*) in Western Germany from 1958 to 1998. Wildlife Biology 16(1):56-65.

Kötsche W, Gottschalk C (1990) Krankheiten der Kaninchen und Hasen. Gustav Fischer Verlag, Jena (4th edition).

Koutsogiannouli EA, Moutou KA, Stamatis C, Walter L, Mamuris Z (2014) Genetic variation in the major histocompatibility complex of the European brown hare (*Lepus europaeus*) across distinct phylogeographic areas. Immunogenetics 66:379-392.

Lewis FI, Otero-Abad B, Hegglin D, Deplazes P, Torgerson PR (2014) Dynamics of the force of infection: insights from *Echinococcus multilocularis* infection in foxes. PLoS Negl Trop Dis. 20;8(3):e2731.

Müller N, Zimmermann V, Forster U, Bienz M, Gottstein B, Welle M (2003) PCR-based detection of canine *Leishmania* infections in formalin-fixed and paraffin-embedded skin biopsies: elaboration of a protocol for quality assessment of the diagnostic amplification reaction. Vet. Parasitol. 114: 223-229.

Ohbayashi M, Rausch RL, Fay FH (1971) On the ecology and distribution of *Echinococcus* spp. (Cestoda: Taeniidae), and characteristics of their development in the intermediate host. II. Comparative studies on the development of larval *E. multilocularis* Leuckart, 1863, in the intermediate host. Jpn J Vet Res 19 [Suppl 3]:1-53.

Rehmann P, Gröne A, Gottstein B, Völlm J, Sager H, Janovsky M, Bacciarini LN (2005) Detection of *Echinococcus multilocularis* infection in a colony of cynomolgus monkeys (*Macaca fascicularis*) using serology and ultrasonography. J Vet Diagn Invest. 17:183-186.

Schantz PM, Lord RD (1972) *Echinococcus* in the South American red fox (*Dusicyon culpaeus*) and the European hare (*Lepus europaeus*) in the Province of Neuquen, Argentina. Ann Trop Med Parasitol 66:479–485.

Scharf G, Deplazes P, Kaser-Hotz B, Borer L, Hasler A, Haller M, Fluckiger M (2004) Radiographic, ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. Vet Radiol Ultrasoun/Offic J Am Coll Vet Radiol Int Vet Radiol Assoc 45(5):411–418.

Schmidt NM, Asferg T, Forchhammer MC (2004) Long-term patterns in European brown hare population dynamics in Denmark: effects of agriculture, predation and climate. BMC Ecology 4:15

Thakur AS, Eddi CS (1982) Ciclo selvatico de la hidatidosis y su importancia zoonotica en los paises latinoamericanos. Gaceta Veterinaria 44:539-543.

Vogel H (1957) *Echinococcus multilocularis* of South Germany. I. The tapeworm stage of strains from humans and animals (in German). Z Tropenmed Parasitol 8:404–454.

Xiao N, Li TY, Qiu JM, Nakao M, Chen XW, Nakaya K, Yamasaki H, Schantz PM, Craig PS, Ito A (2004) The Tibetan hare *Lepus oiostolus*: a novel intermediate host for *Echinococcus multilocularis.* Parasitol Res. 92:352-353.

Zellweger-Fischer J, Kery M, Psinelli G (2011) Population trends of brown hares in Switzerland: The role of land-use and ecological compensation areas. Biological Conservation 144:1364–1373.

Figure 1: Histological presentation of the fertile parasitic lesions recovered from the hare's liver. A: HE-stained section (x200), arrow no. 1 shows the germinal layer and no. 2 an intravesicular protoscolex; B: PAS-stained section (x200), arrow no. 3 pointing at a protoscolex with a few internal hooks, arrow no. 4 shows the PAS-positive laminated layer. Bar is 100 µm.