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ORIGINAL PAPER

Echinococcus multilocularis in south-eastern Europe (Romania)

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Abstract *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis (AE) in humans, has been found in 4.8% of 561 red foxes originating from various regions of Romania. Infected foxes were identified in 8 of 15 counties with average prevalence rates between 1.7% and 14.6%. In previous studies, *E. multilocularis* was not found in 535 foxes from three counties, but larval stages (metacestodes) were present in four species of rodents. Furthermore, AE was diagnosed in two patients. Experiences from other European regions indicate that several factors (such as increasing fox populations with higher parasite prevalences and urban cycles of the parasite) may result in an increased infection risk for humans.

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Introduction

In Europe, the endemic area of *Echinococcus multilocularis,* the causative agent of alveolar echinococcosis (AE) in humans, covers parts of the western continent (France, Benelux States) and all countries of central Europe, including the Baltic States. Furthermore, foci exist in Denmark and on the Norwegian Svalbard Island (Eckert and Deplazes 2004; Romig et al. 2006). In Eastern Europe, Russia belongs to the endemic area, and the parasite has also been found in Byelorussia, Ukraine, Moldavia, and Armenia (Table 1). These data suggest that the European endemic region is connected with the one in northern Asia stretching to the Far East (Bessonov 2002; Eckert et al. 2000; Eckert and Deplazes 2004; Romig et al. 2006).

Most of the epidemiological studies on *E. multilocularis* in foxes or rodents have been performed in the last two decades. Former investigations on fox parasites were not focussed (thematically and methodically) on the detection of *E. multilocularis* and, therefore, these investigations have to be interpreted with caution regarding the infection status with this parasite. However, the few historical reports of *E. multilocularis* metacestode infections from eastern, central-eastern, and south-eastern Europe in humans or animals convincingly document the historical presence of this zoonotic parasite in this area (Table 1) (Kolárová 1999). This seems also to be the case in Lithuania which has experienced a significant increase in the number of AE cases, but the epidemiological data available do not allow

Table 1 Reports of *Echinococcus multilocularis* infections in definitive or intermediate hosts and of alveolar echinococcosis in humans in eastern, central-eastern, south-eastern Europe and in Turkey

Country	Year	Host	Reference
Poland	1995–2006	Fox	Malczewski et al. 1995, 2008; Dubinsky et al. 2006; Kolárová 1999
	1955–2007	Human	Kolárová 1999; Malczewski et al. 2008
Slovenia	2006–2007	Human	Logar et al. 2007
Hungary	1988	Human	Sréter et al. 2003
	2003, 2008–2009	Fox	Sréter et al. 2003, 2004; Casulli et al. 2010
Slovakia	1999, 2001	Fox	Dubinsky et al. 1999, 2001
	2009	Dog	Antolova et al. 2009
	2000–2008	Humans	Kincekova et al. 2008
Bulgaria	1980	Rodents	Genov et al. 1980
Turkey	1934, 1939	Humans	Uysal and Paksoy 1986; Altintas 2003
	1965	Fox	Altintas 2003
Greece	1978	Human	Kern et al. 2003; Theodoropoulos et al. 1978
Moldavia	1961	House mouse	Bessonov 2000, 2002
Ukraine	1957, 2006, 2008	Fox	Bessonov 2000, 2002; Varodi et al. 2006; Kharchenko et al. 2008
Armenia	1958	Human	Gevorkian 1958
Byelorussia	1957, 1958	Rodents	Bessonov 2002
	2001, 2003	Fox	Shimalov and Shimalov 2001, 2003
Lithuania	2003, 2009	Muskrat	Mažeika et al. 2003, 2009
	2005–2006	Dog, pig	Bružinskaitė et al. 2009
	1997–2006	Human	Bružinskaitė et al. 2007
	2001–2004	Fox	Bružinskaitė et al. 2007
Latvia	2008	Fox	Bagrade et al. 2008
Estonia	2005	Fox	Moks et al. 2005
Western part of Russia	1957	Fox	Bessonov 2002
	1966	Wild cat	Bondareva 1966
	1970, 1972, 1998	Fox and human	Lukashenko et al. 1970; Lukashenko 1971, 1972; Bessonov 1998

drawing the conclusion that this parasite only recently had expanded to this area (Bružinskaitė et al. 2007).

Little information exists on the epidemiological situation in south-eastern Europe, including Romania. In this country, *E. multilocularis* was for the first time detected in rodents during studies performed between 1991 and 1995 in the subalpine region of the East Carpathian Mountains (counties Harghita [HR] and Covasna [CV], Fig. 1). The metacestode stage of the parasite was identified by morphological criteria and the presence of protoscoleces. Among 2,416 rodents investigated, the infection rates were as follows: *Microtus nivalis* (*n* 442), 0.45%, *Microtus arvalis* (*n* 120), 0.44%, *Arvicola terrestris* (*n* 1,172), 0.51%, and *Myodes* (syn. *Clethrionomys*) *glareolus* (*n* 120), 1.67% (Sikó 1992, 1993; Sikó et al. 1995). However, until 2002 *E. multilocularis* was neither detected in 485 red foxes from one of the study areas (Covasna) nor in 50 foxes from three other counties (Brasov, Cluj, and Mures) (Gherman et al. 2002). Transmission of the parasite to

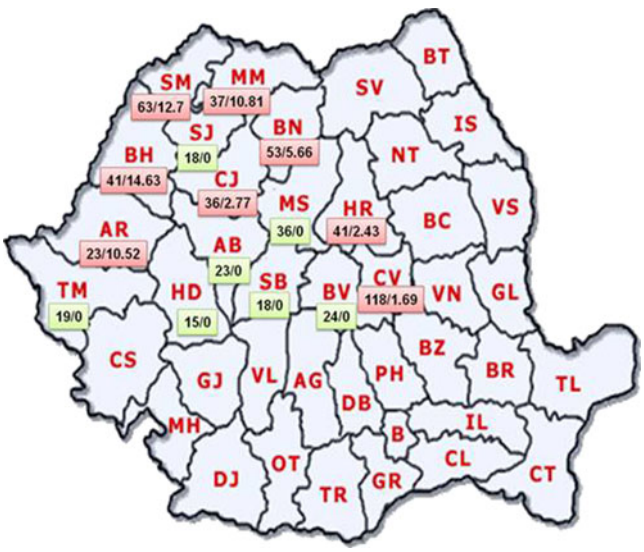

Fig. 1 Geographical distribution of *Echinococcus multilocularis* in Romania: indicated are fox numbers investigated/prevalences (percent)

humans was documented by two cases of AE in patients that originated from north-western Romania (Bihor [BH] county, see Fig. 1) (Panaitescu and şi Pop 1999) and from the central part of the country (Sibiu [SB] county) (Savlovschi 2000), respectively. In neighboring Bulgaria, several species of rodents were found infected with metacestodes of *E. multilocularis* (Genov et al. 1980; Kolárová 1999).

In view of the infection risk posed by *E. multilocularis* to humans, we have performed a new study on the occurrence of the parasite in foxes in Romania.

Material and methods

From August 2007 to March 2010, 561 carcasses of hunted red foxes (*Vulpes vulpes*) (hunting period yearly between October and March) and of animals killed by cars or found dead, were collected from 15 Transylvanian counties (Fig.1). The small intestines were examined for *E. multilocularis* by the intestinal scraping and/or the sedimentation and counting techniques (Hofer et al. 2000). For safety reasons, the intestinal samples were stored at −80°C for 2 weeks before further processing. All tapeworms, including *Echinococcus*, were rinsed in water and stored in 10% formalin for 24 h for microscopical examination, and in selected cases, partially in 95% ethanol for molecular examination. The identification of *E. multilocularis* was based on morphological criteria, including the sack-like form of the uterus in the terminal gravid proglottis and the position of the genital pores (for details, see WHO/OIE 2001). To confirm the morphological identification of *E. multilocularis*, a multiplex PCR (Trachsel et al. 2007) followed by sequence analysis was performed with three isolates from three foxes.

Results

E. multilocularis was identified in 27 (4.8%) of 561 foxes investigated. The morphological diagnosis of *E. multilocularis* was confirmed by molecular analyses in all three isolates from individual foxes.

The geographic distribution of *E. multilocularis* in Romania and the corresponding prevalences are presented in Fig. 1 and Table 2 on a county level. The highest *E. multilocularis* prevalences of 10.5–14.6% were found in the counties bordering Hungary and the Ukraine. Country-wide, there was a tendency of decreasing prevalences towards the central parts.

In addition, high prevalences of other helminths were detected in the 561 foxes investigated (*Alaria alata*, 15.0%, *Dipylidium caninum*, 10.0%, *Mesocestoides* spp., 40.1%, *Taenia* spp., 35.3%, *Trichuris vulpis*, 27.2%, *Ancylostoma caninum*, 18.2%, *Uncinaria stenocephala*, 15.0%, and *Toxocara canis*, 34.0%).

Discussion

The presence of *E. multilocularis* in Romania has been described before in rodents and in two human cases (Panaitescu and şi Pop 1999; Savlovschi 2000; Sikó 1992, 1993; Sikó et al. 1995), but this is the first systematic

Table 2 Prevalences of *Echinococcus multilocularis* in foxes in Romania as assessed by examination of the small intestines

County (abbreviation)	Total no. of examined red foxes	Positive for *E. multilocularis*	Prevalence (%) (95% CI)
Alba (AB)	23	0	0.0 (0.0–12.2)
Arad (AR)	19	2	10.5 (1.3–33.1)
Bihor (BH)	41	6	14.6 (5.6–29.2)
Bistrita (BN)	53	3	5.7 (1.2–15.7)
Brasov (BV)	24	0	0.0 (0.0–11.7)
Cluj (CJ)	36	1	2.8 (0.1–14.5)
Covasna (CV)	118	2	1.7 (0.2–6.0)
Harghita (HR)	41	1	2.4 (0.1–12.9)
Hunedoara (HD)	15	0	0.0 (0.0–18.1)
Maramures (MM)	37	4	10.8 (3.0–25.4)
Mures (MS)	36	0	0.0 (0.0–8.0)
Satu Mare (SM)	63	8	12.7 (5.6–23.5)
Salaj (SJ)	18	0	0.0 (0.0–15.3)
Sibiu (SB)	18	0	0.0 (0.0–15.3)
Timisoara (TM)	19	0	0.0 (0.0–14.6)
Total	561	27	4.8 (3.2–6.9)

investigation and documentation of this zoonotic parasite in the fox population in large parts of the country. Whereas in previous studies, *E. multilocularis* was not recorded from fox intestines using the sedimentation method and morphological examinations of parasite stages (Gherman et al. 2002), the parasite has been found in this study in foxes from 8 of 27 counties (Table 2).

It is well documented that red foxes are the principal definitive hosts of *E. multilocularis* in Europe and are mainly responsible for the contamination of the environment with eggs infective to humans, and occasionally to some other accidental hosts, such as pigs and dogs (Eckert et al. 2000; Eckert and Deplazes 2004; Romig et al. 2006). In recent years, information has accumulated that in some European regions, the infection pressure caused by *E. multilocularis* eggs is increasing due to several factors including increasing fox populations and higher parasite prevalences. Increasing fox populations as observed in many European countries (Switzerland, Germany, Poland, Czech Republic, and others) are regarded as a consequence of successful rabies control by vaccination (Romig et al. 2006). Significant increases of *E. multilocularis* prevalences in foxes during the last decades have been reported from areas in Germany (Berke et al. 2008). Further, a positive correlation between fox densities and parasite prevalences was observed in Switzerland and Germany (Eckert et al. 2000), but the reasons for this phenomenon are still obscure. The numeric increase of fox populations and of parasite prevalences has an effect on the number of human AE cases as indicated by data from Switzerland (Schweiger et al. 2007). Also, the establishment of fox populations in cities during recent years and the development of urban cycles of the parasite have increased the risk for human health.

According to expert opinions, countries or areas with documented endemic occurrence of *E. multilocularis* in definitive hosts should be regarded as risk areas for humans, considering that AE is a rare but severe and potentially lethal disease (Eckert et al. 2000; Eckert and Deplazes 2004). The findings of our study revealing prevalence rates up to 14.6% in foxes which might increase in the future as discussed above are indicators for an existing infection risk for humans in Romania. Therefore, health authorities should initiate at least a minimum of countermeasures, including continuous surveillance of the epidemiological situation, the registration of human cases, and education on preventive measures.

The documentation of *E. multilocularis* infections in foxes in Romania is also of interest regarding the south-eastern range of the parasite's geographical distribution. In Central Europe, the alpine crest seems to mark the rough boundary of the southern distribution. *E. multilocularis* has recently been found in single endemic loci in the southern alpine regions in Switzerland and Italy, but a spread to the lowlands or further to the south was not observed (Manfredi et al. 2006; Tanner et al. 2006). In contrast, a few studies provided supportive evidence that the range of the parasite extends further south in south-eastern Europe, including parts of Romania and Bulgaria, with continuation to Turkey where cases of human AE (diagnosed between 1934 and 1983) have been reported from the European Marmara Region (Uysal and Paksoy 1986). Single cases of human AE have been described from Greece (Kern et al. 2003; Theodoropoulos et al. 1978). Evidently, the epidemiological situation of *E. multilocularis* in south-eastern Europe including Romania requires further attention and additional epidemiological studies.

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