

Spreading of multilocular echinococcosis in southern Europe:
the first record in foxes and jackals in Serbia, Vojvodina Province

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Received: 17 March 2016 / Revised: 16 August 2016 / Accepted: 13 September 2016
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Abstract Foxes and jackals from the Vojvodina Province of Serbia were examined for the presence of echinococcosis. Animals were collected as part of routine rabies monitoring and autopsied; their intestines were checked for parasites. Out of 112 examined foxes, echinococcosis was found in 20 (17.9 %); of 28 examined jackals, 4 were infected (14.3 %). Morphological analysis confirmed the presence of *Echinococcus multilocularis*. This is the first record of *E. multilocularis* in foxes or other carnivorous mammals in Serbia.

Keywords *Echinococcus multilocularis* · Fox · Jackal · Serbia · Vojvodina

Introduction

Foxes (*Vulpes vulpes*) and jackals (*Canis aureus*) are the most important carnivorous mammals on the Balkan Peninsula. In previously fox-populated territory, the number of jackals has

dramatically increased, owing to their habitat plasticity and opportunistic feeding habits, especially around the Danube River (Šálek et al. 2014). Arnold et al. (2012) and Trouwborst et al. (2015) report on the expansion of their range into western and central Europe. In Serbia, jackals have expanded throughout Vojvodina and into western Serbia, with northeast Serbia and lower Srem still considered centres of their dispersal (Paunović et al. 2008). In these circumstances, it is interesting to follow the diseases of carnivorous mammals, especially those with zoonotic potential, of which multilocular echinococcosis is one of the most dangerous.

The Vojvodina Province of Serbia, which represents the southern part of the Pannonian Basin, is geographically open towards central Europe, but is enclosed on the West and South by the large Danube and Sava rivers, and on the East by the Carpathian Mountains. As a consequence, the range of the red fox freely extends from the central parts of Europe into the Pannonian basin, and populations of foxes in Vojvodina reflect changes that occur in the central European fox population. In the last 2 years, Vojvodina has been a rabies-free territory, after great success in oral vaccination of foxes. However, multilocular echinococcosis has not yet been recorded in this host in Serbia (Ćirović et al. 2015; Ilić et al. 2016). The first case of multilocular echinococcosis was recently recorded in Serbia, from a beaver (Ćirović et al. 2012). With respect to this issue, over the last 2 years, we investigated multilocular echinococcosis in foxes and jackals in Vojvodina.

Material and methods

Vojvodina Province covers 21,506 km² of northern Serbia. It is an agricultural region, more than 80 % of its territory

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represented by fertile land, with deciduous forests present in protected areas such as the national park “Fruška gora” mountain.

Adult foxes were collected as part of routine rabies diagnostic procedure in the Pasteur Institute of Novi Sad, National Reference Laboratory for rabies. From 2014 to the winter of 2015–2016, we examined 112 foxes (53 from Srem, 40 from Bačka and 19 from Banat, the three geographic regions of Vojvodina). In addition to this, we examined 28 jackals (25 from Srem, 2 from Bačka and 1 from Banat). After autopsy, intestines were washed and parasites collected and determined morphologically. The intestinal scraping technique was performed, according to Eckert et al. (2001). Recovered parasite individuals were fixed in 10 % formalin and mounted in Canada balsam.

Echinococcus multilocularis was identified on the basis of overall size, size of the last proglottid, body length and last proglottid ratio and the shape of the uterus in the final mature segment (Eckert et al. 2001; Jones and Pybus 2001; Taira et al. 2003; OIE Terrestrial Manual: Manual of diagnostic tests and vaccines for terrestrial animals mammals and birds and bees 2008).

Results

Examination of 112 foxes showed that 20 individuals were infected with echinococcosis (prevalence 17.9 %, 95 % confidence interval 11.46–26.25). Mean intensity of infection was 75.90 (42.05–162.30), with the number of individual tapeworms per host ranging from 1 to 540. Of the 28 examined jackals, four were infected with *E. multilocularis* (P%—14.3 (5.04–31.9); MI—23 (4–56.5)).

Diagnosis was established based on the anterior position of the genital pore and sac-like uterus (Fig. 1). Hook size ranged from 0.025 to 0.035 mm, whereas body length spanned from 1.33 to 3.37 mm. Gravid proglottids were 0.615–1.767 mm in length.

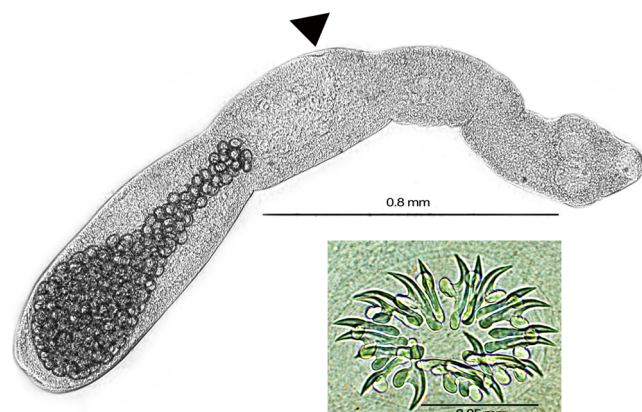


Fig. 1 Adult *Echinococcus multilocularis* from fox, with sac-like uterus with eggs; arrowhead indicates genital pore; inset picture: hooks

The foxes carrying the parasite appeared to be relatively localized geographically, especially in the Vrdnik-Irig-Ruma municipalities of the Srem area, which yielded the highest number of infected hosts and the highest intensities of *E. multilocularis* infection (Fig. 2). Outside of Srem, infection occurred in one fox from Banat, which carried a single tapeworm, and two foxes from Bačka from which 15 and 50 *E. multilocularis* individuals were recovered. Out of four infected jackals from the Srem area, three were from Irig and carried 1, 5 and 70 *Echinococcus* tapeworms. It should be noted that the small size of the Bačka and Banat jackal samples may have influenced the results.

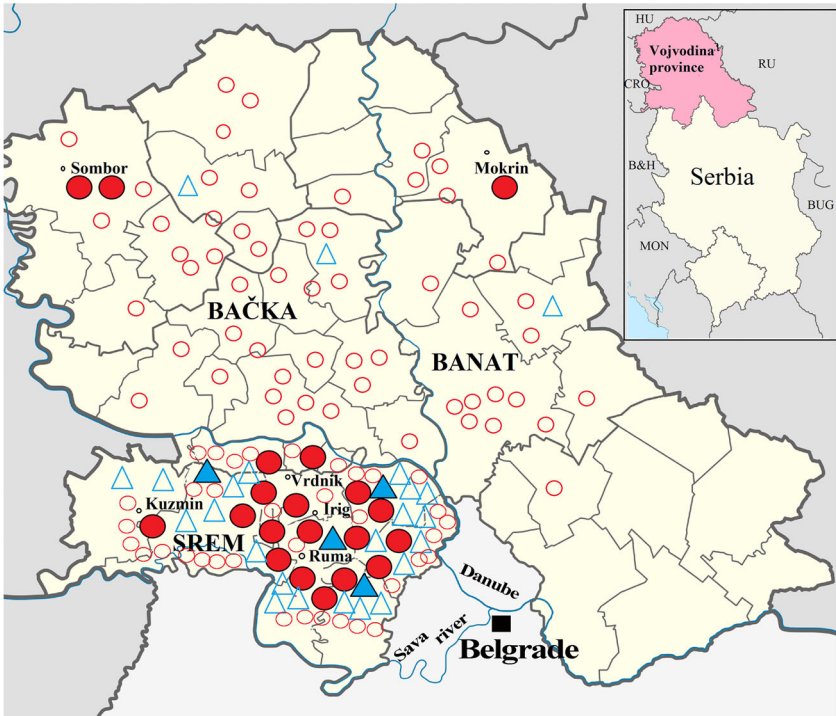
Discussion

The present data confirms the existence of an *E. multilocularis* hotspot in the Vojvodina Province. Considering the spread of the parasite into southern Serbia, as well as our reported prevalence values around 20 %, it can be concluded that there is a stable, enzootic infection present. Sixteen of the twenty infected foxes were from the Irig-Ruma-Vrdnik area, as well as three of the four infected jackals. Relatively high values of mean infection intensity are an additional cause for concern. The only confirmed finding of *E. multilocularis* in Serbia prior to this study was that of Čirović et al. (2012) in a Eurasian beaver (*Castor fiber*) in central Serbia, which originated from a protected colony in the Zasavica nature reserve near the Sava river, previously imported from Germany.

Up until the 1980s, the endemic area of this tapeworm was considered to encompass France, Switzerland, Austria, Germany and Russia. However, data from numerous other European countries refuted this claim. Prevalence of *E. multilocularis* varied from as low as 0.3 % in Denmark (Saeed et al. 2006) and 0.6 % in Great Britain (Smith et al. 2003), up to 31 % in Slovakia (Miterpakova et al. 2009) and 46.3 % in Switzerland (Reperant et al. 2007). Karamon et al. (2015) report increasing prevalences in certain areas of Poland, as well as in Germany, France and the Netherlands.

Detailed studies carried out in Hungary show a higher proportion of infected foxes in the northern parts of the country, and correlate infection with environmental conditions (Casulli et al. 2010; Tolnai et al. 2013). Tolnai et al. (2013) found higher levels of fox infection in areas with permanent water bodies, as well as grassy and arable lands, the same habitats that intermediate hosts such as *Arvicola terrestris*, *Ondatra zibethicus* and *Microtus arvalis* thrive in. Since all of the aforementioned landscape features naturally exist in Vojvodina, the spread of the disease is a possibility. Jackals were also found to be infected in Hungary (Széll et al. 2013). Sikó et al. (2011) published data on the occurrence of this tapeworm species in southeastern Europe, with emphasis on fox infection in Romania, another neighbouring country.

Fig. 2 Map of Vojvodina Province showing the distribution of examined foxes (circles) and jackals (triangles). Filled shapes indicate *Echinococcus multilocularis* infected animals, with its concentration at Fruška gora mountain



Infection of urban foxes is yet another cause for concern, and has been reported in residential and recreational areas in cities such as Zurich, Geneva and Stuttgart (Hofer et al. 2000; Deplazes et al. 2004).

Our current findings, along with data cited above, show that *E. multilocularis* is spreading beyond its initial, relatively limited range, and that monitoring of the helminth fauna of foxes and jackals, as well as rodents which act as intermediate hosts, should be carried out regularly.

Acknowledgments This paper was partially covered by Grant No. TR31084, Ministry of Education and Science of Serbia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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