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RESEARCH NOTE

First identification of *Echinococcus multilocularis* in golden jackals in Croatia

Magda Sindičić, Miljenko Bujanić*, Iva Štimac, Franjo Martinković, Nikolina Tuškan, Marina Špehar and Dean Konjević

Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

Abstract

Alveolar echinococcosis, caused by the tapeworm *Echinococcus multilocularis*, is one of the world's most dangerous zoonosis and an emerging disease with growing incidence in humans. The disease has been reported in new areas and host species in the last two decades, and the primary hosts of the parasite – red fox, golden jackal and grey wolf – are expanding their distribution in Europe. Here we report the morphological and molecular identification of *Echinococcus multilocularis* tapeworms in one of 29 carcasses of adult golden jackals in Croatia, where the only previous report of the parasite was in red foxes in 2016. These results suggest that alveolar echinococcosis should be treated as an emerging disease in Croatia.

Keywords

Echinococcus multilocularis, alveolar echinococcosis, *Canis aureus*, emerging disease

Human alveolar echinococcosis, one of the world's most dangerous zoonosis, is caused by the tapeworm *Echinococcus multilocularis*. Infection begins after consumption of parasite eggs and the larval form known as metacestode causes tumor-like lesions, primarily in the liver, during an asymptomatic incubation period lasting 5–15 years. If untreated, the disease is usually fatal. *E. multilocularis* is restricted to the northern hemisphere, including Europe, Middle East, Russia, Central Asia, northern Japan and North America; 91% of reported cases occur in China (Torgerson *et al.* 2010). Within Europe, the parasite was previously restricted to the central region, but it has expanded to 21 European countries over the past two decades (Oksanen *et al.* 2016).

E. multilocularis has an indirect life cycle that includes carnivores as definitive hosts (*Vulpes vulpes*, *Vulpes lagopus*, *Nyctereutes procyonoides*, *Canis aureus*, *Canis lupus*, *Canis lupus familiaris*, *Felis catus*) and small rodents as intermediate hosts (Oksanen *et al.* 2016). In Europe, the most important definitive host is the red fox (*Vulpes vulpes*), in which it occurs with a prevalence of up to 80% (Konig and Romig 2010). In 2013, Széll *et al.* reported the first *E. multilocularis* infection of golden jackal (*Canis aureus*) in Europe, and those authors predicted that infected vagrants might spread the parasite from Hungary to non-endemic neighbors Croatia and Serbia. Indeed, *E. multilocularis* was confirmed in jackals and foxes in Serbia (Lalošević *et al.* 2016) in 2016, following a report in beaver (*Castor fiber*) imported from Germany in 2012 (Ćirović *et al.* 2012). In Croatia, the parasite has been reported recently for the first time and so far only in red foxes (Beck *et al.* 2018), leading us to screen for its presence in golden jackals. During the last decades jackals are significantly spreading their distribution in Croatia, also inhabiting suburban areas and are intensively hunted and therefore handled by hunters, so they should be considered a possible source of infection for humans and dogs.

A total of 29 complete carcasses of adult golden jackals killed in traffic accidents or culled during regular game management activities (Fig. 1) were collected and stored at –20°C. Large and small intestines were opened longitudinally, flushed with water and sedimented; intestinal contents were examined in Petri dishes under a stereomicroscope at magnifications of 10-45X. Mucosa was scraped, and the scrapings were checked

*Corresponding author: miljenko.bujanic@vef.hr

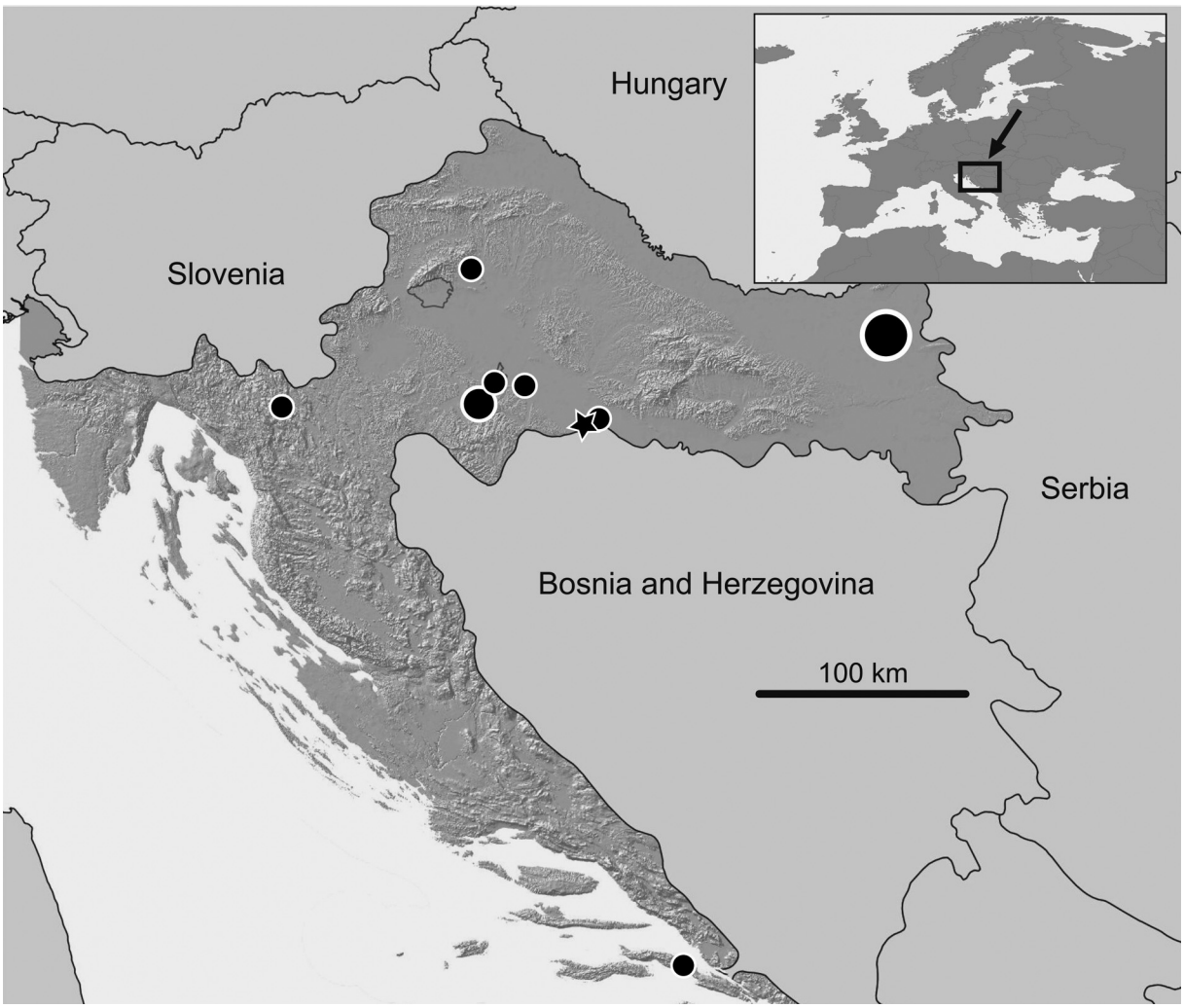

Fig. 1. Geographical distribution of analyzed golden jackal samples. Size of the dot corresponds to the number of samples, star represent the location of *E. multilocularis* positive jackal

for parasites under a stereomicroscope at magnifications of 40-100X. *Echinococcus* sp. tapeworms were isolated, washed with phosphate-buffered saline to remove residual gastrointestinal contents and identified based on morphology (Jones and Pybus 2010) and molecular analysis as described below. Feces collected from the rectum were analyzed using the method of flotation in saturated $ZnSO_4$ solution (specific gravity, 1.3).

DNA was extracted from isolated parasites using a Wizard Genomic DNA Purification Kit (Promega, USA). The large subunits (*rrnL*) of the mitochondrial ribosomal RNA (rRNA) gene were amplified by PCR using primers rrnL-F and rrnL-R (Boubaker *et al.* 2016) and GoTaq® Hot Start Colorless Master Mix (Promega, USA) as described (Boubaker *et al.* 2016). Amplicons were purified and sequenced by Macrogen Europe; sequences were aligned using BioEdit (Hall 2004) and compared with GenBank sequences.

Parasitological examination of intestines confirmed that one animal was infected with four *E. multilocularis* tapeworms. Unfortunately, all the recovered tapeworms had three segments without a gravid one, and rostellar hooks were absent. The parasites were distinguished from *E. granulosus* based on the position of the genital pore in segments: in *E. granulosus*, the genital pore is usually positioned posterior to the midpoint of the segment's lateral margin, whereas the pore in our isolates was located anterior to the midpoint, consistent with *E. multilocularis.* Coprological examination revealed no teniid eggs. A 226-bp *rrnL* rRNA sequence amplified from our isolates matched nucleotides 11,064 – 11,291 of the complete mitochondrial genome of *E. multilocularis* (Genbank AB018440, Nakao *et al.* 2002). Sequence from our study was deposited in the GenBank under accession number MF069153.

The same area where *E. multilocularis* positive jackal from our study originates from, was identified as emerging hot spot by Beck *et al.* (2018) in their study of *E. multilocularis* presence in red foxes. Also, this location is about 250 km away

from the Serbian province of Vojvodina, where Lalošević *et al.* (2016) identified 14.3% prevalence of the parasite in golden jackals. The Croatian golden jackal population over the last 15 years has significantly increased in size and distribution presumably due to immigration from Bulgaria, Romania and Serbia (Fabbri *et al.* 2014), so these immigrant may be the source of infection. Other explanations are also possible, maybe the parasite was present in the country earlier but detected only recently because of improvement in diagnostics (Conraths and Deplazes 2015) and increased wildlife surveillance (Torgerson *et al.* 2010).

Spreading of *E. multilocularis* into Croatia is part of a global trend of parasite expansion into new areas and host species over the last two decades, which has helped drive growing incidence of alveolar echinococcosis in humans (Schweiger *et al.* 2007; Carmena and Cardona 2014). Indeed, within Europe, the parasite's primary hosts – red fox, golden jackal and grey wolf – are expanding their distribution (Deplazes *et al.* 2004; Kaczensky *et al.* 2013; Trouwborst *et al.* 2015). But parasite expansion can only partially be explained by changing dynamics of host populations (Guerra *et al.* 2014). Also, intermediate hosts such as *Microtus arvalis* play an important role in maintaining the parasite (Guerra *et al.* 2014).

The results from our study and from previous work (Beck *et al.* 2018) suggest that alveolar echinococcosis should be treated as an emerging disease in Croatia, and that all fox, jackal and wolf carcasses submitted for obligatory rabies testing should also be checked for *E. multilocularis*. Proper surveillance must be a priority of Croatian veterinary authorities, since countries that conduct wildlife disease surveillance are more likely to detect infectious and zoonotic disease and swiftly adopt countermeasures (Mörner *et al.* 2002).

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