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Echinococcus multilocularis in a Eurasian lynx (*Lynx lynx*) in Turkey

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Research Article

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Abstract

Echinococcus multilocularis is the causative agent of alveolar echinococcosis (AE), one of the most threatening zoonoses in Eurasia. Human AE is widespread in the Erzurum region of Turkey, but the situation of the disease in intermediate and definitive hosts is unknown. A Eurasian lynx (*Lynx lynx*) was killed in a traffic accident in the north of Erzurum, and was taken to our laboratory. Sedimentation and counting technique (SCT), DNA isolation and polymerase chain reaction (PCR) analysis were performed. The SCT results showed that the lynx was infected with *E. multilocularis* with a medium (745 worms) worm burden. The DNA of adult worms obtained from the lynx was analyzed with a species-specific PCR, and the worms were confirmed to be *E. multilocularis* by 12S rRNA gene sequence analysis. This is the first report of *E. multilocularis* from Eurasian lynx in Turkey.

Introduction

Wildlife has received increasing attention in recent years as a potential reservoir of diseases for domestic animals and humans. Wild animals are also affected by pathogenic agents that spill over from domestic animal hosts (Thompson, 2013).

Echinococcus multilocularis is the causative agent of alveolar echinococcosis (AE), one of the most threatening zoonoses in Eurasia and a potentially fatal disease if untreated. The parasite is characterized primarily by its sylvatic life cycle, although peridomestic or synanthropic cycles may also occur in some geographic areas (Romig *et al.* 2017). *Echinococcus multilocularis* has a holarctic distribution, extending longitudinally from North America to Eurasia (Deplazes *et al.* 2017).

The life cycle of *E. multilocularis* predominantly involves predators (wild canids) as definitive hosts and many species of rodents, common canid prey, as intermediate hosts (Eckert *et al.* 2011; Hegglin *et al.* 2015; Umhang *et al.* 2016). Foxes are thought to be the main sources of environmental contamination with eggs of the parasite in most endemic areas in Europe, but infections have also been found in coyotes, raccoon-dogs, wolves, jackals and occasionally wild cats. Domestic dogs and, to a lesser extent, cats can be involved in the transmission cycle. However, the role of other species in maintaining the *E. multilocularis* cycle is unknown (Kapel *et al.* 2006; Hegglin and Deplazes, 2013; Conraths and Deplazes, 2015).

Parasite detection at necropsy and subsequent identification following specific morphological criteria are generally used for the postmortem detection of *Echinococcus* adult worms in wild carnivore definitive hosts. The sedimentation and counting (SCT) technique is regarded as the gold standard method (Eckert *et al.* 2001; Conraths and Deplazes, 2015). The diagnostic sensitivity and specificity of SCT are accepted to be 83.8 and 100%, respectively (Conraths and Deplazes, 2015). Molecular techniques like polymerase chain reaction (PCR) are frequently used as a confirmatory test and also for phylogenetic analysis to identify the genetic differences between geographically different isolates (Deplazes *et al.* 2003; Carmena and Cardona, 2014). Besides, in recent years copro-PCR analysis are commonly used by researchers as a diagnostic test for echinococcosis (Trachsel *et al.* 2007; Dyachenko *et al.* 2008; Boufana *et al.* 2013; Knapp *et al.* 2016*a*).

Echinococcus multilocularis prevails in the northern hemisphere, comprising Central Europe, Russia, Central Asian republics, northern Japan, parts of North America and some countries of the Middle East (Torgerson *et al.* 2010; Deplazes *et al.* 2017). Turkey is a common site for human AE, with an estimated annual incidence of 100 cases per year (Torgerson *et al.* 2010). The disease primarily occurs in the eastern Anatolian region, especially in Erzurum province (Altintas, 2003). The studies regarding the definitive hosts of *E. multilocularis* are neglected in Turkey. The first report of *E. multilocularis* in Turkey was recorded by Merdivenci (1963) from a fox in the northwest of Turkey. The second report was by Avcioglu *et al.* (2016) from a fox in Erzurum province of Turkey. In Erzurum, the largest number of human cases was detected so far, but the disease ecology in intermediate and definitive hosts is unknown. This study describes the infection of adult *E. multilocularis* in a Eurasian lynx (*Lynx lynx*) from Turkey.

Materials and methods

A lynx was found killed by a traffic accident on the highway Erzurum-Artvin (40°17′6.50″N–41°33′23.75″E), approximately 47 km north of Erzurum, Turkey (Fig. 1a) in December, 2016.

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Fig. 1. *Echinococcus multilocularis* isolated from the Eurasian lynx. (A) Lynx, (B) mature *E. multilocularis* worms (C) a mature worm (blue arrows: genital pore).

The sexually mature male feline was taken to our laboratory. The stomach and intestines of the lynx were collected and placed in labelled zip-top bags, stored at −86 °C for 7 days. Then, the intestines were stored at −20 °C until further examination.

The stomach and intestines were examined macroscopically, and observed parasites were collected. The parasites were identified based on their morphological characteristics. The sedimentation and counting technique (SCT) was performed to identify the presence of *Echinococcus* spp. as defined by Hofer *et al.* (2000). Numerous worms were found; thus, an aliquot was collected and the total parasite count was estimated from the proportion of the aliquot to the total sediment. The intensity of infection was classified according to Duscher *et al.* (2005). Adult worms of *E. multilocularis* were differentiated using morphological characteristics (size, length of gravid proglottids, the position of the genital pore and shape of the uterus) by light microscopy and stereomicroscopy.

DNA was isolated from adult worms using a DNA extraction kit (G-spin™ Total DNA Extraction Kit; Intron, Korea) according to the manufacturer's instructions. The obtained DNA was stored at −20 °C. A target sequence in the mitochondrial 12S ribosomal RNA (rRNA) gene was amplified by direct PCR using species-specific primers (Dyachenko *et al.* 2008). Distilled water was used as the negative control and DNA of *E. multilocularis* obtained from a fox (GenBank: KU711929) was used as the positive control.

Bidirectional sequencing was performed with an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA) using the ABI PRISM® BigDye terminator cycle sequencing kit. The sequence determined was then subjected to nucleotide BLAST (blast.ncbi.nlm.nih.gov). Sequences were edited and aligned using Bioedit 7.0. (www.mbio.ncsu.edu/BioEdit/bioedit.html).

Results and discussion

The Eurasian lynx (*Lynx lynx*) is widely distributed in Asia and Europe. The distribution and status of lynx in Turkey are missing data. The fragmentation of forest, lack of prey species, illegal hunting and car crashes represent significant threats to this species. Little is known about the distribution and ecology of the Eurasian lynx in eastern Turkey (Chynoweth *et al.* 2015). The Eurasian lynx eats a wide range of prey including ungulates, gamebirds and small mammals. But, they generally prey on small mammals especially rodents in our region.

In this study, macroscopic examination of the stomach and intestines revealed that the lynx was infected with *Mesocestoides* spp. and *Toxascaris leonina* parasites. No *Taenia* spp. was found. Also, 9 rodents were found in the stomach of the lynx.

The SCT results showed that the lynx was infected with *E. multilocularis* with a medium (745 worms) worm burden (Fig. 1b). Eighty-five percent of the worms were mature and they were

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morphologically identified as *E. multilocularis*. Immature worms smaller than 1.5 mm were also observed. The adult worms measured approximately 2.25 (1.82–2.95) mm in length, and the mature strobila had 3–4 proglottids, with the final one gravid. The length of the gravid proglottids was less than half of the body length. The lateral genital pore was above the midportion of the proglottids (Fig. 1c). Egg production was observed in only very few parasites with very low egg numbers. Approximately, 12% of the eggs were fully developed containing an oncosphere.

The adult worms obtained from the lynx were molecularly analysed with species-specific PCR and were confirmed to be *E. multilocularis* by 12S rRNA gene sequence analysis. The sequence determined in this study was deposited in GenBank under the accession number KY039185. The sequence was aligned and compared with some related sequences in GenBank. The sequence of *E. multilocularis* from the lynx showed 100% identity with those of European *E. multilocularis* from Poland (KF171966, KR229987 and KR229985), UK (JX068642) and Germany (EU043372, L49455 and KP941429). The sequence was also identical to the previously published ones in Turkey from fox (KU711929) and human (KX099963-6 and KX664080-6), whereas the sequence similarity was 99.5% as compared with those of the isolates from Japan (AB031351 and AB024424) and Russia (KX185950-2).

In this study, the presence of *E. multilocularis* in a Eurasian lynx from Erzurum, a region where AE is common, was determined by microscopy and confirmed by PCR-based sequencing. Apart from our study, Pomamarev *et al.* (2011) have been reported *E. multilocularis* infection of *Lynx lynx* from the Altai. There is also one report on the presence of *E. oligarthra* in a bobcat (*Lynx rufus)* (Salinas-López *et al.* 1996) in Mexico. In contrast to *E. multilocularis*, adult worms of *E. oligarthra* accepted to be specifically adapted to wild felids (Romig *et al.* 2017).

Generally, the life cycle of *E. multilocularis* includes fox and dog as definitive hosts and cricetid rodents as intermediate hosts. In endemic areas, cats also can be definitive hosts. In addition, if the infection prevalence is higher in rodents this will result in detection of the infection in different definitive hosts like in our study. In a recent study, Avcioglu *et al.* (2017) reported *E. multilocularis* infection in rodent intermediate hosts in Erzurum with a prevalence of 1.3%. All of the infected rodents had fertile metacestodes. Since the main prey of the lynx is rodents in this region, this can explain the *E. multilocularis* infection in the Eurasian lynx. Also, it is possible to come across with *E. multilocularis* infection in different wild carnivores eating rodents in their diet in this region.

Cats are accepted to be less susceptible to infection with *E. multilocularis* than dogs (Kapel *et al.* 2006). However, there are studies reporting the presence of *E. multilocularis* with a prevalence between 0 and 5.5% in various endemic areas in domestic cats (Jenkins and Romig, 2000; Gottstein *et al.* 2001; Thompson *et al.* 2003; Nonaka *et al.* 2008; Eckert *et al.* 2011; Knapp *et al.* 2016*b*). Umhang *et al.* (2015) reported *E. multilocularis* infection from one European wildcat. The parasite infection in cats usually displays very low numbers of gravid worms and a markedly reduced egg production of these worms. Thompson *et al.* (2006) indicated that in cats, worm population maturated and produced thick-shelled eggs but their overall development was retarded in their experimental studies. High worm burdens were also reported, but the infection comprised only immature worms without eggs, supporting the opinion that cats play an insignificant role in *E. multilocularis* transmission, even in a 'highly endemic' region (Hegglin and Deplazes, 2013; Umhang *et al.* 2015). In consequence, felids are considered as less suitable definitive hosts to maintain the parasite cycle and are of minor zoonotic relevance (Kapel *et al.* 2006; Nonaka *et al.* 2008; Otranto *et al.* 2015).

In conclusion, in this study, we report that a Eurasian lynx was infected with *E. multilocularis* with a burden of 745 worms, including mostly mature and immature worms. Only a few of the parasites showed egg production, with minimal egg numbers. Approximately, 12% of the eggs were fully developed containing an oncosphere. Further studies are needed to evaluate the infectivity of these eggs and the role of lynxes as a definitive host.

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Conflicts of interest. The authors have no conflicts of interest to declare.

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