

**Co-infection with *Angiostrongylus chabaudi* and *Dirofilaria immitis* in a wildcat, *Felis silvestris* from Romania – a case report**

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**Abstract**

*Dirofilaria immitis* is a worldwide spread nematode affecting the pulmonary artery and the heart of dogs (rarely reported in cats), especially in areas where the dogs show a high prevalence of infection. *Angiostrongylus chabaudi* is, in turn, a cardiopulmonary nematode of felids identified in several southern European countries and Germany. Co-infections of *Dirofilaria immitis* and *Angiostrongylus* spp. are known only in canids. We report a case of *D. immitis* and *A. chabaudi* co-infection in a road-killed wildcat originating from Southeastern Romania. Overall, 17 nematodes were collected from the pulmonary arteries of the wildcat and were morphologically identified as *A. chabaudi* (2 males and 15 females). Another nematode was collected from the right ventricle and identified as adult male *D. immitis*. Genomic DNA was extracted from one nematode of each of the two species and a fragment of the *cox1* gene was amplified and sequenced. The sequences obtained from the nematodes showed 100% similarity to a sequence of *A. chabaudi* isolated from Romania (Accession number KU521521) and to various *D. immitis* sequences from Europe, Asia and Australia (e.g. KT716014, EU159111, AJ537512). This is the first report of *A. chabaudi* and *D. immitis* co-infection in a felid, revealing the possibility of similar situations in domestic cats. This requires a more in-depth clinical and laboratory examination of animals with respiratory and cardiac symptoms.

*Wildlife, lungworms, feline parasitology*

The wildcat, *Felis silvestris* is a small feline found in the Old World characterized by a broad diet that includes micromammals, birds, fish, reptiles, arthropods, and gastropods (Lanszki et al. 2016), making it susceptible to the infection with a wide variety of parasites (Krone et al. 2008). Moreover, their crepuscular and nocturnal lifestyle exposes wildcats to mosquito bites. Among the parasitic diseases, cardio-pulmonary nematodes can cause severe diseases in both domestic and wild felids worldwide. Several species of cardio-respiratory parasites have been found in felids, including *Aelurostrongylus abstrusus*, *Troglostrongylus brevior*, *T. subcrenatus*, *Eucoleus aerophilus*, *Angiostrongylus chabaudi*, and *Dirofilaria immitis* reported from various regions and with variable prevalence (Traversa and Di Cesare 2014).

*Angiostrongylus chabaudi* is a snail-borne nematode localized in the pulmonary arteries and right heart of domestic and wild cats and has a limited geographic distribution: Italy (Biocca 1957; Traversa et al. 2015), Germany (Steeb et al. 2014), Greece (Diakou et al. 2016), Romania (Gherman et al. 2016), and Bulgaria (Giannelli et al. 2016). The filarioid mosquito-borne nematode *D. immitis* infects the heart and pulmonary arteries of carnivores, primarily canids, but it is rarely reported in cats (McCall et al. 2008). Its limited occurrence in cats has been explained mainly by the enhanced host resistance, most of the infections being recorded mainly in hyper-endemic regions where about 10–25% of the dogs are infected (Venco et al. 2011).

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Co-infections of *D. immitis* and *Angiostrongylus* spp. are rarely reported in carnivores. Such co-infections are known only in canids in which *D. immitis* - *Angiostrongylus vasorum* co-infections were reported in domestic dogs in Portugal (Alho et al. 2014) and Italy (Del Prete et al. 2015; Di Cesare et al. 2015), and in a golden jackal in Serbia (Gavrilović et al. 2017). To our knowledge, so far there have been no reported co-infections by *D. immitis* and *A. chabaudi* in felids, a situation which we report in the current paper.

### Case presentation

A road-killed male wildcat was collected in Soveja (45.9919795°N; 26.6358592°E), Vrancea County, Romania. The body was necropsied, and parasites located in the right ventricle and the pulmonary arteries were preserved in formalin for morphological characterization, and in ethanol for molecular analysis. The species was identified using morphological keys and descriptions (Biocca 1957; Costa et al. 2003; Furtado et al. 2010). For further confirmation of the species identification, molecular tools were also used. Genomic DNA was extracted from both species of nematodes (one individual from each), using a commercial kit (Isolate II Genomic DNA Kit, Bioline, UK). The molecular characterization was performed by PCR amplification of a portion of the *cox1* gene. For *A. chabaudi*, a 710 bp fragment was amplified using the universal invertebrate primer pair, LCO1490/HCO2198, as previously described (Folmer et al. 1994). For *D. immitis*, a 670 bp portion was amplified using the Spirurida-specific NTF/NTR primer pair, according to previously published protocols (Casiraghi et al. 2001). The amplicons were submitted to direct sequencing, using an external service (Macrogen Europe).

Overall, 18 nematodes were collected from the pulmonary arteries and the right ventricle of the wildcat (Plate III, Fig.1). Of these, one nematode was identified as adult male *D. immitis* and 17 specimens were identified as *A. chabaudi* (2 males and 15 females). In the case of *A. chabaudi* females, the body (18.3–23.5 mm length, 0.245–0.296 mm width) had a barber-pole aspect. The males (14.5–15.0 mm length; 0.189–0.221 mm width) were uniformly coloured and had a small copulatory bursa with two symmetrical lateral lobes and a poorly developed dorsal lobe. All the features were in line with previous morphological descriptions of the species (Biocca 1957; Gherman et al. 2016; Diakou et al. 2016). The male *D. immitis* (142.6 mm in length; 0.43 mm in width) had a spiralled posterior end with a smooth cuticle, except the caudal extremity, which presented parallel cuticular ridges. Two unequal spicules were present on the lower curvature, the larger one (0.35 mm) placed in a canal of the smaller one (0.18 mm). The paired and unpaired pre-, ad-, and post-papillae were also visible. All other characteristics were in accordance with the known features of the species (Furtado et al. 2010).

The sequence obtained from the nematodes morphologically identified as *A. chabaudi* showed 100% similarity to a sequence of *A. chabaudi* previously isolated from Romania (Accession number KU521521). Similarly, the sequence obtained from the nematode morphologically identified as *D. immitis* showed 100% similarity to various *D. immitis* sequences from Europe, Asia and Australia (KT716014, EU159111, AJ537512).

### Discussion

Both species identified in this study are generally rarely reported in felids. *Dirofilaria immitis*, a typical canine parasite, has been previously reported in wildcats only in Serbia (Penezić et al. 2014) and Romania (Ionică et al. 2017). In domestic cats, the reports are also scarce, and found mainly in areas where prevalence of the infection in dogs is high (McCall et al. 2008). The scarcity of *D. immitis* reports in felids was explained by the poor suitability of cats as definitive hosts (McCall et al. 1992). In cats, only

a small percentage of worms become adults and their lifespan is no longer than 2–4 years, with most worms dying before reaching maturity (McCall et al. 2008). Although microfilariaemia is uncommon in infected cats, DNA of microfilarial origin was found in one out of ten wildcats examined in Romania (Ionică et al. 2017). Another hypothesis for the low prevalence of *D. immitis* in felids is related to the feeding preferences of the mosquito vectors (Labarthe et al. 1998). On the other hand, for *A. chabaudi*, a typical felid parasite, the scarcity of reports is probably related to misidentification of faecal larvae with other lungworm species or to poor surveillance (Gherman et al. 2016).

The wildcat from this case report originated from Vrancea County, Southeastern Romania. To date, no infection with *D. immitis* was found in this county in dogs or wild canids, but there have been reports of infection in other canids in neighbouring counties (Ionică et al. 2017). No information is available about the natural gastropod hosts of *A. chabaudi*. Recently, *Cornu aspersum* snails have been experimentally confirmed as a suitable intermediate host for *A. chabaudi* (Colella et al. 2017). However, gastropods are rarely part of the wildcats' diet (Lanszki et al. 2016), suggesting that their infection with *A. chabaudi* could be, as in the case of *A. vasorum* (Spratt 2015), related to the consumption of paratenic hosts (i.e. birds, reptiles or amphibians).

Generally, co-infections are known to be more clinically severe than mono-infections. The clinical importance of co-infections with cardiopulmonary nematodes in cats was highlighted by Traversa et al. (2015) who diagnosed a domestic cat infected with three nematode species: *A. abstrusus*, *T. brevior*, and *A. chabaudi*. This is also the only description of the clinical presentation of an animal infected with *A. chabaudi*. To the best of our knowledge, this is the first report of *A. chabaudi* and *D. immitis* co-infection in a felid and highlights the possibility of similar situations in domestic cats, which requires a more in-depth clinical and laboratory examination of animals with respiratory and cardiac symptoms.

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