Angiostrongylus chabaudi in felids: New findings and a review of the literature

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Abstract

Cardiopulmonary infections by *Angiostrongylus chabaudi* affect domestic and wild felids but, due to limited information on the biology of this nematode, its pathogenicity remains unclear. This article describes the histopathological alterations associated with *Angiostrongylus* infection in a wildcat from Bulgaria, and reviews current literature on this feline angiostrongylid. Nematodes were isolated from lung lavage and faecal samples of a road killed wildcat in Southern Bulgaria. The morphological identification of parasite larvae as *A. chabaudi* was confirmed by molecular analysis of part of the 18S ribosomal RNA gene. Upon histopathological examination, severe granulomatous pneumonia, ranging from multifocal to coalescing, and pulmonary vascular lesions were observed. Extensive alveolar collapse, alveolar emphysematous changes, parenchymal haemorrhages and small artery wall hyperplasia were observed in the parenchyma adjacent to the granulomas. Histopathological examination revealed the presence of cross-sections of adult female parasites within the lumen of the pulmonary artery branches, the intima altered markedly by subendothelial proliferation and oedematous changes. This study compliments current knowledge of the pathogenesis of feline angiostrongylosis by *A. chabaudi* in wildcats, as well as of the distribution of this little-known parasite.

1. Introduction

The superfamily Metastrongyloidea includes 181 roundworm species, ranked into 46 genera, which affect the cardiopulmonary and circulatory systems of several vertebrates, including cetaceans, marsupials, rodents, ruminants, and carnivores (Spratt, 2015). With the exception of a few species, such as Filaroides hirthi or Oslerus osleri, whose life cycle is direct (McGarry and Morgan, 2009), the transmission of metastrongyloids generally occurs via gastropod intermediate hosts, in which larval development occurs from first (L1) to the infective third stage larva (L3) (Anderson, 2000). Gastropods, containing the L3s, can be ingested by paratenic hosts (e.g., rodents) (Jeżewski et al., 2013, Cowie, 2013), which are in turn preyed on by felid definitive hosts; alternatively, definitive hosts may become infected by accidentally ingesting infected gastropods (Cowie, 2013, Helm et al., 2015, Lesage et al., 2015), or their mucus trails (Giannelli et al., 2015b, Colella et al., 2015). Upon infection of a definitive host, lungworm larvae migrate through the animal's body, until they reach the respiratory tract, where they develop into adult nematodes. Depending on the species, adult stages may localize in the respiratory system (i.e., from the trachea to the alveolar ducts), pulmonary arteries, or mesenteric veins (Anderson, 2000). Metastrongyloidea display a high degree of definitive host-specificity, developing only in selected groups of animals, with the exception of zoonotic Angiostrongylus species of rodents (Anderson, 2000, Spratt, 2015). In addition, the French heartworm Angiostrongylus vasorum (Strongylida, Angiostrongylidae) specifically infects canids (dogs and foxes), but can also develop in immunodepressed cats experimentally inoculated with L3s (Guilhon and Cens, 1970, Dias et al., 2008).

The renewed interest in metastrongyloids of domestic cats has spurred new research on these parasites and, particularly, on the potential negative impact that the infection may exert on wildlife (Traversa and Di Cesare, 2013, Giannelli et al., 2016). Indeed, generally, data on non-zoonotic parasites of wildlife attract limited interest, thus generating fragmentary basic information on their biology in the definitive host, even if these are considered endangered species (Thompson et al., 2010, Jenkins et al., 2015). This is particularly true for metastrongyloids of felids, whose basic biology is scantily described in isolated reports (i.e., nematodes recovered from animals killed during poaching or roadside casualties) (Diakou et al., 2016, Gherman et al., 2016) or over the course of sporadic surveys (Krone et al., 2008, Falsone et al., 2014, Steeb et al., 2014, Napoli et al., 2016, Veronesi et al., 2016). Nevertheless, it is currently believed that domestic and wild felids worldwide are threatened by Aelurostrongylus abstrusus (Strongylida, Angiostrongylidae) (Elsheikha et al., 2016) and Troglostrongylus spp. (Strongylida, Crenosomatidae) lungworms; the latter have been increasingly reported in several (albeit confined) foci, both in the Old and New worlds (Brianti et al., 2014b). In addition, infections by Oslerus rostratus (Strongylida, Filaridae) have been sporadically reported in felids from Israel, Italy, Spain, United States and Sri Lanka (Brianti et al., 2014a). Conversely, information on nematodes of the genus Angiostrongylus affecting felines have gained new visibility only recently. Indeed, a new species, Angiostrongylus felineus (Strongylida, Angiostrongylidae), has been recently detected in the eyra cat Herpailurus yagouaroundi (Vieira et al., 2013), whilst Angiostrongylus chabaudi (Strongylida, Angiostrongylidae) has been increasingly reported in domestic and wildcats from Italy (Varcasia et al., 2014, Traversa et al., 2015, Veronesi et al., 2016), Greece (Diakou et al., 2016), Romania (Gherman et al., 2016), and Germany (Steeb et al., 2014). However, despite these reports, the fundamental biology of this nematode is still unclear and several questions concerning the distribution, life cycle, and pathogenicity of A. chabaudi remain unanswered. Similar to A. vasorum (Schnyder et al., 2010), adult specimens of A. chabaudi localize to the right side of the heart and pulmonary arteries of the definitive host (Biocca, 1957); thus, it is plausible that these parasites might equally impact the physiology of the cardiopulmonary system of infected animals. In this article, we describe the first case of A. chabaudi infection in a wildcat from Bulgaria, as well as the histopathological findings at the site of nematode localization. In addition, we discuss our observations in light of currently available knowledge of feline angiostrongylosis and metastrongylosis in Europe.

2. Materials and methods

On January 2016, an adult male, road killed wildcat was found at ∼15 km north (42°33′N; 25°37′E) of Stara Zagora (Southern Bulgaria). The animal was identified as a pure European wildcat Felis silvestris silvestris based on morphological and morphometric features (Krüger et al., 2009). The wildcat was necropsied, and the upper respiratory tract and intestines were isolated and dissected for parasite detection. Lungs were soaked in saline solution for 24 h and, subsequently, the sediment was analysed for the presence of metastrongyloid larvae and/or adults, as previously described (Giannelli et al., 2014a, Olsen et al., 2015). Concurrently, faeces collected from the rectal ampulla were examined using the Baermann technique (Giannelli et al., 2015a). In addition, lungs were fixed in a 10% buffered formalin solution for histological examination. Serial sections obtained from the left and right lobes and through the derivation of the pulmonary artery were stained with haematoxylin and eosin (H&E) (Giannelli et al., 2014a).

Nematodes detected in the lung and Baermann sediments were mounted on microscope slides with saline, examined, photographed and measured using an optical microscope (Leica® DLMB2) equipped with LAS AF 4.1 software. Morphological identification was based on key features described in previously published articles (Diakou et al., 2016, Gherman et al., 2016, Giannelli et al., 2014b). In addition, for confirmatory molecular identification, genomic DNA from single larvae, isolated from the lungs and Baermann sediment, was extracted using the DNeasy Blood & Tissue Kit (Qiagen, GmbH, Hilden, Germany), in accordance with the manufacturer's instructions. A portion of the 18S ribosomal RNA gene (∼1708 bp) was amplified with primers NC18SF1 (5′-AAAGATTAAGCCATGCA-3′) and NC5BR (5′-GCAGGTTCACCTACAGAT-3′) as described previously (Patterson-Kane et al., 2009). Each reaction consisted of 4 µl genomic DNA (∼100 ng) and 46 µl of PCR mix containing 2.5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.3, 250 µM of each dNTP, 50 pmol of each primer and 1.25 U of Ampli Taq Gold (Applied Biosystems, California, USA). Samples without DNA (negative controls) were included with each batch of samples tested. Cycling conditions were: 95 °C for 10 min (first polymerase activation and denaturation), 35 cycles of 95 °C for 30 s (denaturation), 57 °C for 30 s (annealing) and 72 °C for 1 min (extension), and a final extension at 72 °C for 7 min. All amplicons were resolved in GelRed-stained (2%) agarose (Biotium, California, USA) gels and sized by comparison with markers in the 1 kb DNA Ladder (MBI Fermentas, Vilnius, Lithuania). Gels were photographed using the GelLogic 100 gel documentation system (Kodak, New York, USA). Amplicons were purified and sequenced, in both directions using the same primers as for PCR, employing the Big Dye Terminator Cycle Sequencing Kit (v. 3.1, Applied Biosystems, Foster City, California, USA) in an automated sequencer (ABI-PRISM 377). Sequences were compared with those available in the GenBank database, using Basic Local Alignment Search Tool (BLAST—http://blast.ncbi.nlm.nih.gov/blast.cgi).

3. Results

During the necropsy, no ectoparasites or adult nematodes were detected, including upon examination of the upper respiratory tract and intestines. Conversely, L1s of metastrongyloids were detected in the Baermann and pulmonary sediments. Upon histopathological examination, severe granulomatous foci of pneumonia, ranging from multifocal to coalescing, and pulmonary vascular lesions were observed. The pulmonary inflammation was characterised by the presence of epithelioid cells, giant cells, macrophages and lymphocytes surrounding the eggs and larvae at different stages of development. Extensive alveolar collapse, alveolar emphysematous foci, parenchymal haemorrhages and small artery wall hyperplasia were observed in the parenchyma adjacent to the granulomas (Fig. 1A and B). Eggs and larvae were sequestered inside granulomas, with several observed free within alveolar spaces and lung septa. Cross-sections of adult females were observed within the lumen of the pulmonary artery branches, whose intima was markedly altered by subendothelial proliferation and oedematous changes. Papillary intimal projections and thrombotic material partially occluded the lumens of the pulmonary artery branches, whereas the tunica media of these vessels was markedly thickened and multi-focally fibrotic (Fig. 1A; Fig. 2). Transverse sections of adult parasites measured from 150 to 350 µm in diameter. The parasites were characterised by the presence of coelomyarian musculature arranged perpendicularly to a smooth external cuticle, muscles projecting into the pseusocoelum in a cylinder-like shape, with an evident bright red contractile portion interrupted by small accessory hypodermal chords. The nematodes contained a large intestine composed of multinucleated cells with an ill-defined brush border and multiple sections of ovaries.

The isolated larvae measured 370 ± 13.2 μm in length and 14 ± 1.2 μm in width. The cephalic extremity was rounded, with a terminal buccal opening, whereas the caudal extremity was characterised by a small dorsal spine and notch, ending in a short sigmoid tail (Fig. 3). Based on these morphological and morphometrical features, the parasite was identified as A. chabaudi. BLAST analysis of the partial 18S sequence (accession number KX378963) displayed 100% nucleotide identity to an Angiostrongylus sp. recovered from a wildcat from Germany (accession number KM216825), subsequently identified as A. chabaudi (Varcasia et al., 2014).

4. Discussion

This study provides further information on A. chabaudi infection in wildcats, with new data on the histological alterations and on its diagnosis and differentiation from other parasites affecting the cardiopulmonary system of felids. The life history of A. chabaudi is unknown. This nematode was originally described in wildcats living in the forested areas of central Italy (Biocca, 1957), and more than 50 years later in both domestic and wild felids from other European countries (Varcasia et al., 2014, Veronesi et al., 2016, Diakou et al., 2016, Gherman et al., 2016, Steeb et al., 2014, Traversa et al., 2015). Hence, the distribution of this nematode is wider than previously thought. Based on our observations, it can be argued that wildcats may play an important epidemiological role as the main definitive host of A. chabaudi. From analysis of the literature, reports indicated that the majority of animals affected were less than two years of age, thus indicating that young felids are at high risk of infection. Whether this is associated with their remarkable predatory activity/playing attitude is yet to be determined.

Data on the morphology of A. chabaudi confirms that L1s of this angiostrongylid species are featured by distinctive characteristics. However, while the anatomy of buccal opening and the shape of the caudal extremity are homogeneous among the specimens here examined and those previously studied (Diakou et al., 2016, Gherman et al., 2016), larval length shows wide fluctuations, ranging from 307 to 419.7 μm and 362–400 μm, in specimens examined from Romania and Greece, respectively (Diakou et al., 2016, Gherman et al., 2016). Therefore, larvae of metastrongyloids affecting felids should be identified based on an altogether evaluation of their length and shape of anterior and posterior extremity (Diakou et al., 2016).

Histopathological data confirms the localization of adults of A. chabaudi is the small pulmonary arteries of wildcats (Biocca, 1957). This observation differs from other metastrongyloids affecting felids (Table 1). Indeed, adult nematodes of A. abstrusus, T. brevior and Oslerus rostratus localize within sub-pleural nodules of the lungs (Traversa and Di Cesare, 2013), in the respiratory airways (i.e., trachea, large bronchi, and bronchioles) (Giannelli et al., 2014a, Giannelli et al., 2014b), and to the peri-bronchial tissue between the fascia and the bronchial cartilage, respectively (Brianti et al., 2014a). These differences may have underlying implications for the pathogenicity of the disease. It has been suggested that the severity of symptoms of feline metastrongyloid infections is proportional to the number of L1s shed in the faeces (Genchi et al., 2014). However, the anatomical localization of adult nematodes may be linked to the severity of the clinical presentation. For instance, the presence of adult T. brevior in the small bronchi has been associated with severe catarrhal bronchitis in felids, accompanied by massive catarrhal exudates and emphysematous foci, which obliterate the airways (Giannelli et al., 2014a). Conversely, the confinement of O. rostratus in pseudo-cystic formations surrounded by fibrous tissues may be responsible for the absence of apparent clinical signs during the remissive stage of the infection (Brianti et al., 2014a). In the case of A. chabaudi, the presence of eggs, larvae and adult nematodes in the pulmonary arteries is responsible for severe damage to the vascular system, which histologically appears as subendothelial proliferation and oedema, ultimately leading to the onset of thrombosis. The same lesions have previously been reported in another wildcat infected by A. chabaudi (Diakou et al., 2016), in which marked hypertrophy of the arterial wall was explained to be as a result of the pulmonary hypertension caused by the presence of nematodes in the pulmonary arteries (Diakou et al., 2016). Similarly, dogs and foxes infected with A. vasorum may suffer from the presence of thrombi, associated with the presence of larvae and eggs in pulmonary arteries and arterial wall thickening, along with granulomas consisting of macrophages, multinucleated giant cells and lymphocytes surrounding the parasite (Schnyder et al., 2010, Poli et al., 1991). Considering the close genetic relationships between the domestic cat and the European wildcat (Mattucci et al., 2013, Driscoll et al., 2011), they may present a similar histopatological picture during A. chabaudi infection and, accordingly, a comparable pathogenesis. Based on the lesions observed in this animal, we hypothesize that A. chabaudi may cause life-threatening disease in felids, although current data is not sufficient to establish if populations of F. silvestris are endangered by this parasitic infection. Future studies on a much larger number of live animals are warranted in order to elucidate the biology of this parasite and to describe the clinical alterations it may induce in felids. In the meantime, the susceptibility of other wild felid species to infection should not be discounted, as recently demonstrated for the Eurasian lynx (Lynx lynx) and the caracal (Caracal caracal) to T. brevior and A. abstrusus, respectively (Alić et al., 2015, Di Cesare et al., 2016).

In conclusion, all of the information available on A. chabaudi suggests that wildcats are involved in the transmission of this angiostrongylid. This may indicate that the distribution of this nematode overlaps that of F. silvestris, whose population includes enough individuals to be included as Least Concern by the IUCN red list of threatened species (Yamaguchi et al., 2015). If confirmed, the presence of A. chabaudi in wildcats will inevitably raise questions on the role of this felid in the transmission of the parasite to domestic cats.

Figures

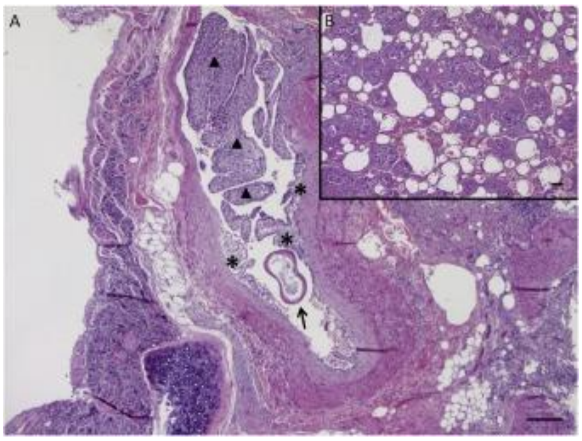

Fig. 1. Histopathology of the pulmonary artery (A) and lung (B). (A) Endoarteritis (asterisks) of the pulmonary artery showing an intraluminal adult nematode (arrow) and thrombotic formations (arrowheads); (B) severe diffuse pneumonitis with granulomas centred on eggs and larvae (H&E stain; scale bar = 200 μm).

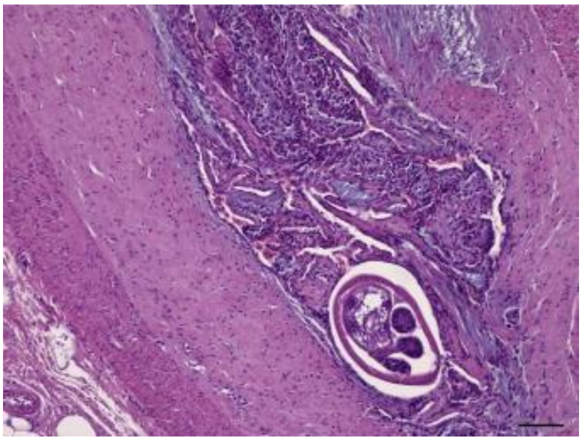

Fig. 2. Histopathology of pulmonary artery branches. Occurrence of thrombotic material partially occluding the vessel lumens, along with transverse sections of adult parasites (H&E stain; scale bar = 100 μm).

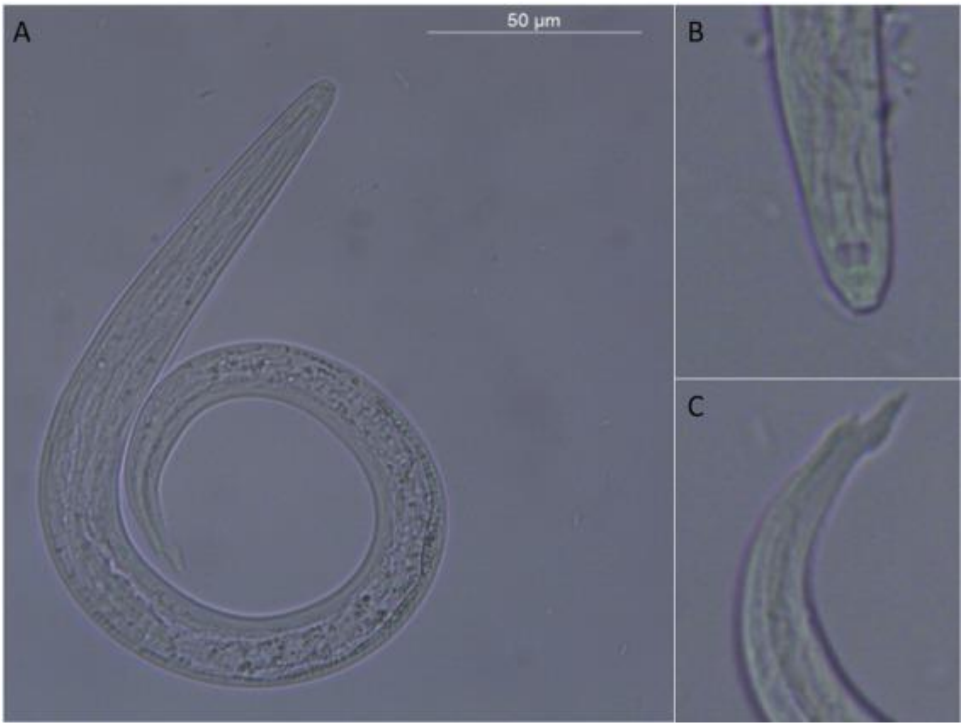

Fig. 3. L1 of *Angiostrongylus chabaudi* (A, scale bar = 50 μm), with details of the anterior (B) and posterior extremities (C).

Table 1

Species	Type host	Other hosts	Anatomical localization	Reference
Aelurostrongylus abstrusus	*Felis catus*	*Acinonyx jubatus, Felis pardalis, Panthera pardus, Panthera tigris, Panthera leo, Felis bengalensis, Felis bengalensis euptilurus, Lynx lynx, Caracal caracal, Leptailurus serval, Oncifelis geoffroyi, Leopardus pardalis, Leopardus colocolo*	Respiratory bronchioles, alveolar ducts	Fiorello et al. (2006), Di Cesare et al. (2015) , Gressler et al. (2016)
Angiostrongylus chabaudi	*Felis silvestris*	*Felis catus*	Pulmonary arteries	Diakou et al. (2016), Varcasia et al. (2014)
Angiostrongylus felineus	*Herpailurus yagouaroundi*	-	Pulmonary arteries	Vieira et al. (2013)
Oslerus rostratus	*Felis catus*	*Lynx rufus*	Peri-bronchial tissues	Brianti et al. (2014a)
Troglostrongylus brevior	*Felis ocreata, Felis chaus*	*Felis silvestris, Felis catus, Lynx lynx*	Bronchi	Alić et al. (2015), Brianti et al. (2014b)
Troglostrongylus subcrenatus	*Panthera pardus, Panthera tigris*	*Felis silvestris, Felis catus*	Trachea, bronchi	Brianti et al. (2014b)
Troglostrongylus troglostrongylus	*Prionailurus bengalensis*	-	Frontal sinuses	Brianti et al. (2014b)
Troglostrongylus wilsoni	*Lynx rufus*	*Felis canadensis*	Bronchi, lungs	Brianti et al. (2014b)

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