

First molecular evidence of *Angiostrongylus vasorum* in an African Golden wolf (*Canis lupaster*) in Algeria

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Abstract

Background

Angiostrongylus vasorum, the “French heartworm” is a nematode belonging to the Metastrongyloidea superfamily. This parasite was first identified in Toulouse, France by Serres in 1853 infecting the pulmonary arteries and the right side of the heart of a Pointer dog. There is no report regarding this nematode in Algeria. This report aims to investigate the presence of lungworms among different mammal species in different Algerian regions.

Materials and methods

Between February 2022 and September 2023, 47 road-killed animals were collected from six departments in Algeria. All carcasses were subjected to a full parasitological investigation, and lung tissues were preserved in 10% buffered formalin and 70% ethanol. All collected samples were subjected to histology and PCR for lungworm identification.

Results

In a single golden African wolf (*Canis lupaster*) collected from Constantine, the histological examination revealed the presence of nematode eggs and larvae in the alveolar space and the interstitium-associated chronic obstructive vascular changes. The molecular identification confirmed the presence of *Angiostrongylus vasorum*. All the other animals were negative for lungworms.

Conclusion

To the best of our knowledge, this is the first report of *A. vasorum* infecting a golden African wolf (*Canis lupaster*), reporting a new host association, highlighting the importance of further studies to update the geographical distribution and its epidemiology across Algeria.

Background

Angiostrongylus vasorum, the “French heartworm” was first identified in Toulouse, France by Serres in 1853 infecting the pulmonary artery and the right side of the heart of a Pointer dog [1]. Since then, the parasites have been described across a wide range of countries, in Europe, America, and Africa [2]. In the last decade, the parasite gained much interest among researchers due to the severe clinical picture in domestic dogs and the fast expansion in Europe [3]. *Angiostrongylus vasorum* is the causative agent of angiostrongylosis in canids, and the symptomatology can vary from subclinical to lethal with the most commonly observed symptoms including breathing difficulty, heart insufficiency, coagulation disorder, anorexia, and lowered physical endurance. Clinical neurological signs are also common and can be

manifested as: ataxia, paresis, paralysis, or seizures [4–6]. Unusual localisations of adult nematodes have been reported in the anterior chamber of the eye [7–8], femoral artery [9], pericardial sac and in the bladder [10]. Larval stages were identified in the brain [11], diaphragm, pancreas, liver, and skin [10].

This nematode exhibits an indirect life cycle using gastropods (snails and slugs) as intermediate hosts, while small vertebrates can act as paratenic hosts [6, 12–14]. The primary transmission route of *A. vasorum* is through the ingestion of third-stage larvae with the intermediate host (intentionally or accidentally) and/or a paratenic host. Furthermore, contamination can occur when the final host consumes food contaminated with infective intermediate hosts [4, 15] or water contaminated with third-stage larvae [16]. There is also the possibility of contamination by consuming free L3 released in snail slime [17, 18].

Among wild animals, wild foxes (*Vulpes vulpes*) commonly serve as the typical definitive hosts for *A. vasorum* [18]. Nonetheless, there have been multiple reports of other wild canid definitive host species such as crab-eating fox (*Cerdocyon thous*), hoary fox (*Lycalopex vetulus*), coyote (*Canis latrans*), golden jackal (*Canis aureus*), grey wolf (*Canis lupus*), and raccoon dog (*Nyctereutes procyonoides*) [4, 12, 19–22]. Non-canid wild captive animals like the red panda (*Ailurus fulgens fulgens*) [23] and meerkats (*Suricata suricatta*) [24] were also confirmed as definitive hosts. All these hosts have the potential to act as reservoirs for the infection for domestic dogs (*Canis familiaris*) [4, 25], and cats (*Felis catus*) [26].

In Africa, the first case was documented in five necropsied domestic dogs from Uganda in 1972 [27] and more recently, a potential autochthonous case in a 6-month-old asymptomatic dog was reported in Morocco based on the morphology of L1 [28].

One hundred mammal species belonging to thirty-seven families and eleven orders occur in Algeria, of which carnivores are represented by twenty-one species and seven families [29]. These species have a diet mainly based on scavenging or predation, making them more exposed to food-borne parasitic infections [30]. Considering the abundant wild fauna in Algeria and the occurrence of the parasite in other northern African countries, this study aimed to investigate the presence of *A. vasorum* in animal hosts in Algeria.

Material and methods

Between February 2022 and September 2023, 47 road-killed animal carcasses belonging to Carnivora (11 *Canis familiaris*, 9 *Canis lupaster*, 6 *Felis catus*, 1 *Genetta genetta*, 3 *Herpestes ichneumon*, 3 *Vulpes vulpes*, and 13 *Vulpes zerda*) and Rodentia (1 *Hystrix cristata*) were collected from 11 localities and 6 departments in Algeria (Table 1) (Fig. 1), then transported to the National Research Center (CRE) in Annaba according to the national legal provisions. Until examination, the carcasses were safely enclosed in an identified plastic bag and stored at -20°C. Each carcass was examined by an extensive parasitological necropsy and comprehensive data regarding the animals' age and sexual maturity were collected according to [31–32]. For all animals, the entire cardio-respiratory system was removed, and the trachea, bronchi, and bronchioles as well as the pulmonary arteries and the heart chambers were

longitudinally opened and carefully examined for parasites using a stereomicroscope as described in [33]. During necropsy, macroscopic pictures of the lungs were taken where lesions were observed and from each animal, samples of lung tissue were collected and preserved in 10% buffered-formalin and concentrated ethanol respectively. Subsequently, the collected samples were legally transported to the Faculty of Veterinary Medicine (University of Agriculture and Veterinary Medicine) of Cluj-Napoca, for molecular and histological analyses.

The formalin-fixed lung tissue samples were trimmed and embedded in paraffin wax, according to standard protocols. Two-three μm thick sections were subsequently stained with haematoxylin and eosin (H&E). The histological assessment was performed using a light Olympus BX-41 microscope connected to an Olympus SP 350 digital camera. The photomicrographs were taken using the Stream Basic imaging software (Olympus Corporation, Tokyo, Japan). When histology showed a parasitic infection, the entire quantity of the solutions in which lungs were conserved was placed in a 15 ml conical centrifuge tube and centrifuged. The obtained sediment was examined using a stereomicroscope. Genomic DNA was isolated from small lung sections of all animals using a commercial kit (Isolate II Genomic DNA kit, meridian Bioscience, London, UK) according to the manufacturer's instructions. Additionally, DNA was isolated from a pool of larvae collected from the solution in which the African wolf lung tissues were conserved. Subsequently, a fragment of the mitochondrial cytochrome c oxidase subunit 1 (cox1, ~ 700 bp) gene was amplified by conventional PCR, using the universal primers LCO1490/HCO2198, according to literature [34]. The PCR product was excised from the gel, purified on a silica membrane spin column (Gel/PCR DNA Fragments Kit, Geneaid Biotech, Taiwan), and sequenced bidirectionally using an external service (performed by Macrogen Europe B.V., Amsterdam, The Netherlands). The sequences were assembled using Geneious software (Biomatters LTD, New Zealand) and compared to other sequences available in the NCBI GenBank® database by Basic Local Alignment Search Tool (BLAST) analysis.

Results

At the time of the necropsy, adult lungworms were not detected in any of the examined animals. In one single African golden wolf (*Canis lupaster*) collected from Constantine (36°21'N 6°36'E), the pulmonary parenchyma showed moderate congestion and oedema, multifocal dark-red and variably-sized areas of densification, compatible with foci of ischemic necrosis, severe haemorrhage, and interstitial verminous pneumonia (Fig. 1a, b). The lesions were mainly located at the periphery of the lung parenchyma. No adult parasites were observed within the right ventricle and pulmonary arteries during the macroscopical and microscopical examination. The lungs of the other examined animals had no serious macroscopic lesions. In the African golden wolf with pulmonary lesions, histological examination revealed numerous transverse to longitudinal sections of nematode larvae and eggs free in the alveolar spaces and interstitium, associated with a mixed inflammatory reaction composed mainly of macrophages, including binucleated and multinucleated cells, plasma cells, small lymphocytes, and a few eosinophils. The larvae were approximately 20–30 μm in width and 80–90 μm in length with a thin basophilic cuticle and numerous deep basophilic, internal nuclei. The eggs were round to ovoid, thin-walled, of approximately 50–60 μm , and contained either a larva or a morula. Additional findings included arterial

thrombosis, proliferative endarteritis with recanalization, pulmonary necrosis, haemorrhage, oedema, interstitial fibrosis, type II pneumocyte hyperplasia, and small aggregates of hemosiderin-laden macrophages (Fig. 2a-d). No important histological changes related to lungworms were noted in the pulmonary parenchyma collected from the other animals. Larvae recovered from the sediment of the centrifuged solutions were morphologically consistent with *Angiostrongylus* spp. larvae (Fig. 3).

The BLAST analysis revealed 99.17–100% nucleotide identity to numerous other *A. vasorum* isolates from dogs and foxes registered in Europe (e.g. OQ210698, GQ982791, GQ982874). The sequence was deposited in GenBank under the Accession Number PP872515.

All the other animals were negative by PCR as well.

Discussion

The current study reports for the first time the presence of *A. vasorum* in Algeria, which is the first molecular confirmation of the species in Africa, and a new host-parasite association report. This observation may indicate the continuous geographical expansion of this parasite to new areas that were previously not considered endemic, as recorded also in Morocco [28]. Interestingly, although the first report of *A. vasorum* in Africa dates back more than 50 years ago, in domestic dogs from Uganda [27], the lack of more recent reports could be attributed to the lack of awareness or interest among veterinarians and dog owners regarding this potentially fatal disease, as noted in previous study [4] or, more probably, to the sporadic occurrence of *A. vasorum* in Africa. Another hypothesis would be that *A. vasorum* was introduced in Northern Africa more recently. In the more recent report from Morocco, *A. vasorum* was identified in a symptomatic domestic dog (*Canis familiaris*) from Rabat city using the morphological identification of L1 collected from faeces. The occurrence of *A. vasorum* is related to several factors: biotic (humidity, temperature) and abiotic factors (intermediate, paratenic, and final hosts). In the study area from Algeria where the carcass was collected numerous species of gastropods were reported [35]. However, the specificity of *A. vasorum* for the intermediate host is broad, so many snails can host the larvae for their development [25]. In endemic areas in Europe, the emergence of this disease could be linked to various factors, such as climate change, urbanization of the red fox, and dog movement [36].

The present finding underlines the importance of complementary diagnostic methods for the detection and identification of lungworms as previously recommended [33]. Once again, histology proved to be a key method for the detection of *A. vasorum* infection [37]. The reason of missing the adult nematodes while performing the necropsy are most likely related to the low number of worms or to the lack of use of additional methods like lung perfusion technique. Examination of faeces using the larval concentration method could also have revealed some larvae although the carcass was frozen [38]. The absence of infection in other animal species might be correlated to the low number of examined animals, their food habits or just their unsuitability as hosts.

In Algeria, there are many stray and free-roaming dogs [39] sharing the same lifestyle (scavenging) as other canids such as the African golden wolf, and could be easily infected with the reported parasite, therefore, further studies are required for a better understanding of the epidemiological scenario of angiostrongylosis and to determine the potential life cycle pattern of *A. vasorum* in Algeria.

Conclusion

This new host and geographical record of *A. vasorum* expands the knowledge on this clinically important parasite, highlighting the importance of studies in wild carnivores from areas where the investigations on their parasitic fauna were historically limited and advocates the use of complementary diagnostic techniques when dealing with dead animal examinations.

Declarations

Ethics approval and consent to participate

The National Environmental Research Center Annaba Ethics Committee granted ethical permission. The Algerian legislation (Ordinance No. 06 – 05 of 19 Jounada Ethania 1427, corresponding to July 15, 2006) was followed, according to the authors, in the collection and examination of the animals. The gathering of carcasses was reported to the local authorities, who also verbally approved the fieldwork activities.

Consent for publication

Not Applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NM; carcasses collection, performed necropsies, wrote the first draft, **GD;** Performed Necropsies, performed the PCRs and revised the manuscript. **AMI;** Performed the PCRs and revised the manuscript. **CGT, AGN, MT;** Performed the histopathological examination and revised the

manuscript. **ZB** and **ADM**; coordinated the study, revised and approved the final version of the manuscript.

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Table 1

Table 1. Animal species included in the study, geographical locations

Department	Locality	Species	Examined animals	Lungworms (N/%)
El Tarf		<i>Canis familiaris</i>	6 (3 M, 3 F)	-
		<i>Canis lupaster</i>	1 M	-
	Ain kerma	<i>Herpestes ichneumon</i>	1 M	-
		<i>Canis lupaster</i>	1 F	+
		<i>Hystrix cristata</i>	1 M	-
	Bouhadjar	<i>Canis lupaster</i>	1M	-
		<i>Vulpes vulpes</i>	2 (1 M, 1 F)	-
		<i>Felis catus</i>	1 M	-
		<i>Herpestes ichneumon</i>	1 M	-
		<i>Canis fammiliaris</i>	1 F	-
Drean		<i>Genetta genetta</i>	1 F	-
		<i>Canis lupaster</i>	1 F	-
	Zitouna	<i>Canis familiaris</i>	1 F	-
		<i>Canis lupaster</i>	2 (1 M, 1 F)	-
		<i>Felis catus</i>	4 (1 M, 3 F)	-
Annaba	Annaba	<i>Herpestes ichneumon</i>	1 M	-
		<i>Canis lupaster</i>	4 (1 M, 3 F)	-
	Seraidi	<i>Canis Lupaster</i>	1 M	-
		<i>Canis lupaster</i>	1 M	<i>Angiostrongylus vasorum</i> (1, 2.13%)
		<i>Vulpes vulpes</i>	1 F	-
Skikda		<i>Felis catus</i>	1 M	-

	Skikda	<i>Canis lupaster</i>	1 M	-
Algiers	Staoueli	<i>Canis familiaris</i>	2 (1 M, 1F)	-
Oued Souf	Oued souf	<i>Vulpes zerda</i>	13 (7 M, 6F)	-
Total			47 (24 M, 23F)	1 M

Figures

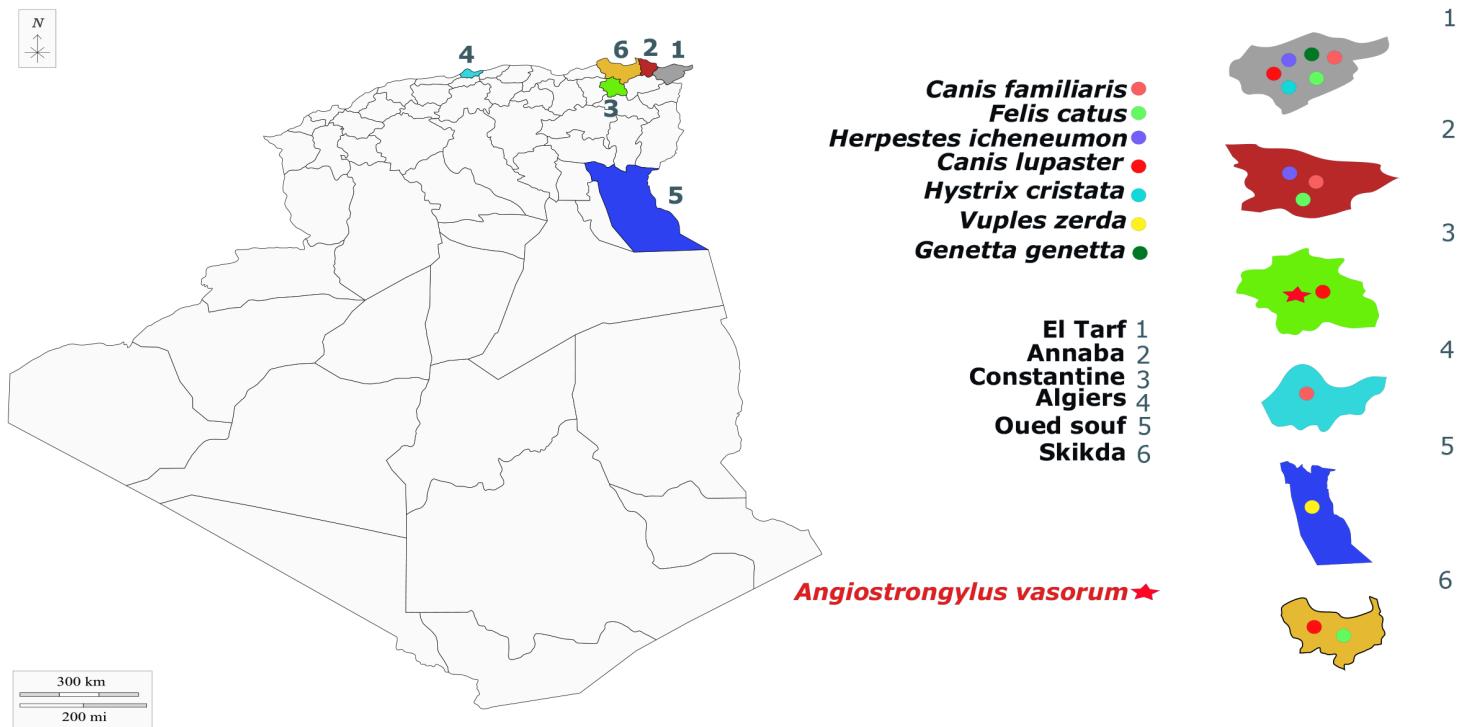


Figure 1

Map showing the geographical locations of the tested animals and the *A. vasorum* positive animal

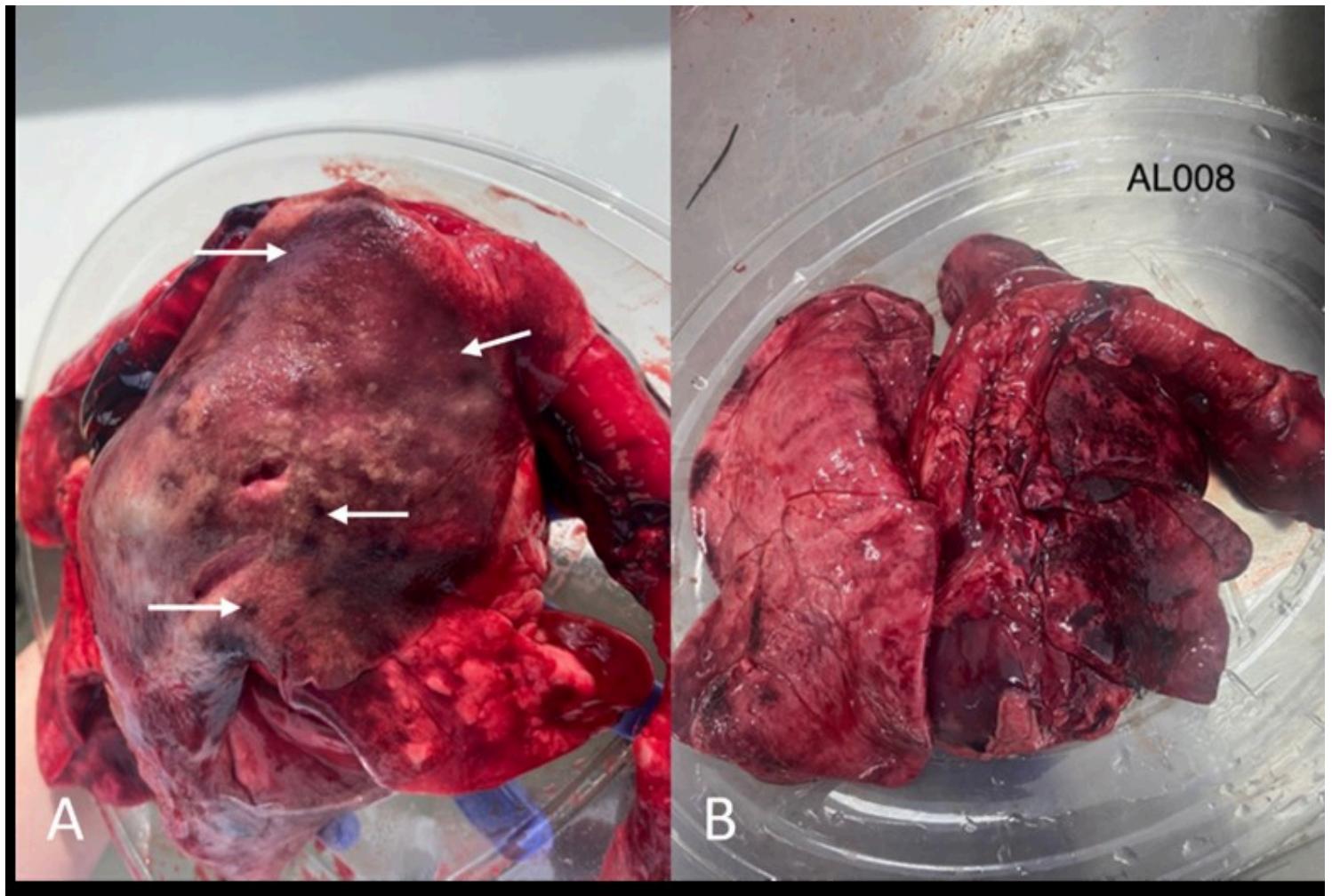


Figure 2

Gross evaluation of the lungs: A, B: Pulmonary parenchyma showing multifocal to coalescing dark-red areas (arrows) consistent with verminous pneumonia and severe alteration of blood vessels.

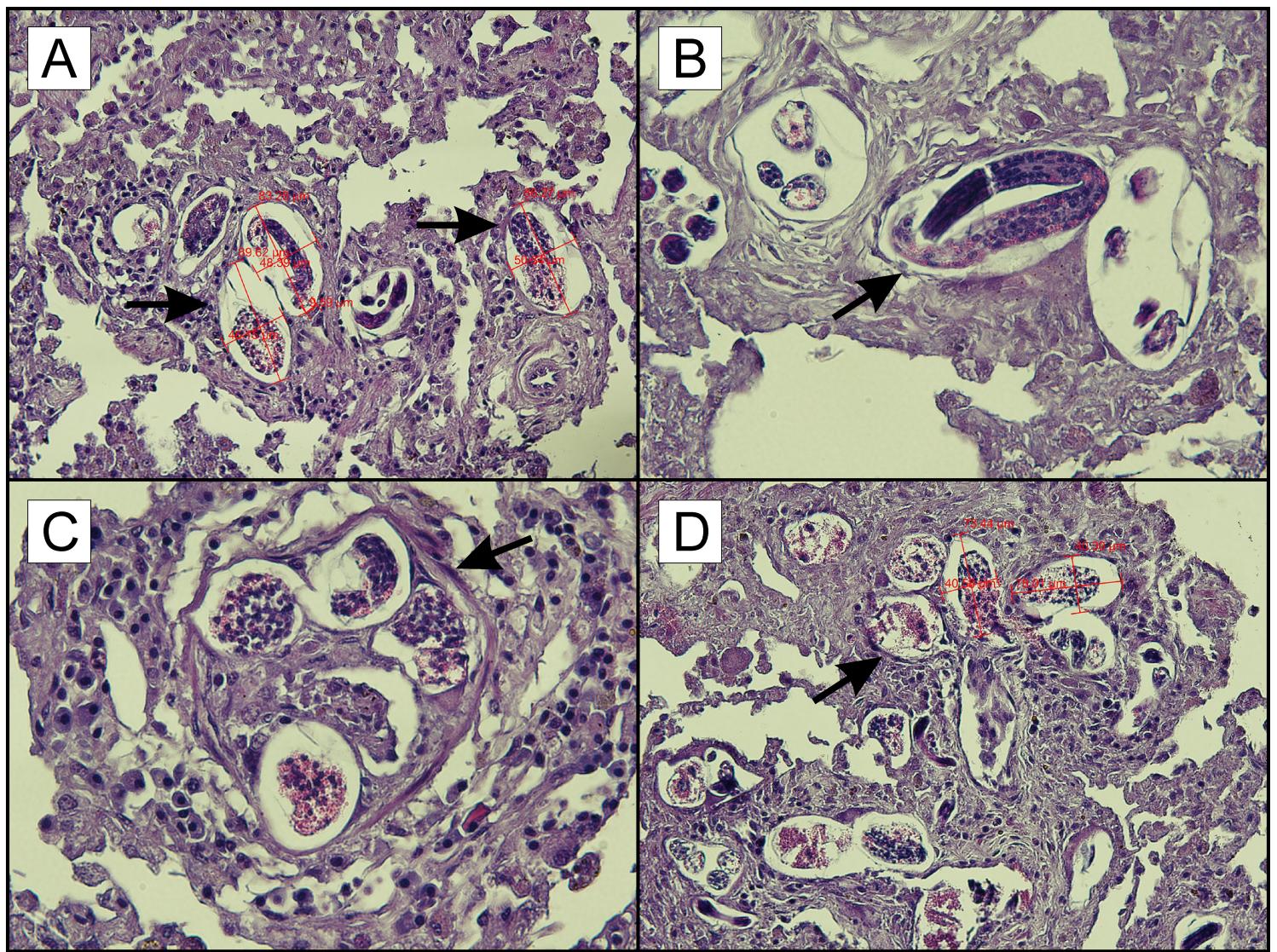


Figure 3

Microscopical findings of pulmonary angiostrongylosis in an African golden wolf (*Canis lupaster*): A-D)
The pulmonary interstitium is moderately expanded by numerous inflammatory nodules centered on parasitic eggs and larvae(arrows). The morphology of the parasitic structures is suggestive for *Angiostrongylus spp.*



Figure 4

First-stage larva of *Angiostrongylus vasorum* collected from the lung. Note the terminal oral opening and the kinked tail with a dorsal spine and a notch

Supplementary Files

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