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Mortality associated with *Angiostrongylus cantonensis* in non-human primates in Europe

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ABSTRACT

Between December 2020 and March 2022, three cases of fatal meningoencephalitis were documented in two red-fronted brown lemurs (*Eulemur rufus*) and a ring-tailed lemur (*Lemur catta*) at the Bioparc in Valencia, eastern Spain. Post-mortem analyses revealed moderate congestion in the meninges of the brain in all cases. Multifocal areas of hemorrhage were observed in one lemur, primarily in the cerebellum and brainstem. Histopathological examination showed mainly acute hemorrhagic and necrotic changes, together with moderate eosinophilic and/or histiocytic meningoencephalitis, with perivascular cuffing, and gliosis. Numerous nematode larvae were found in the meninges, brain, and spinal cord, with or without associated inflammation, hemorrhage, and necrosis. Considering the affected host species, nematode morphology, and its anatomical localization, *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae) was suspected as the causative agent. Phylogenetic studies based on the internal transcriber spacer 1/cytochrome *c* oxidase subunit I (ITS1/COI) genes confirmed the initial suspicion of this zoonotic parasite and its relationship to sequences from the Balearic and Canary Islands. This is the first known detection of *A. cantonensis* in non-human primates in Europe and it represents one of the few occurrences reported in the Palearctic region to date. Further research on this zoonotic parasite is crucial to understanding its spread in Spain, assessing public health risks, and developing effective control measures to mitigate outbreaks and protect human and animal health.

1. Introduction

Angiostrongylus cantonensis (Chen, 1935) (Rhabditida: Metastrongylidae) is an emerging zoonotic parasite that poses significant public health concern. Commonly referred to as the rat lungworm, this metastrongyloid nematode primarily infects rodents, notably the synanthropic species *Rattus norvegicus* and *Rattus rattus*, acting as its definitive host (Kottwitz et al., 2014). Other species such as birds, horses, dogs, and non-human primates (NHPs) can act as accidental hosts (Morgan et al., 2021). All of them, and humans, act as dead-end hosts and typically become infected through the ingestion of infected snails, slugs, or even their slime, which acquire the parasite mainly through the consumption of vegetables contaminated by the feces of infected rats (Rollins et al., 2023). The L3s remain infective in paratenic hosts such as mollusks, crabs, or shrimps (Jarvi and Prociv, 2021). This parasite is now recognized as

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the leading cause of human eosinophilic meningitis worldwide, also known as neuroangiostrongylosis (NAS), which can be life-threatening in some cases (Cowie et al., 2022). In NHPs, the first case of infection was reported in two cynomolgus monkeys (*Macaca fascicularis*) imported from Indonesia to Japan (Kodama et al., 1981). Since then, several other fatal cases have been documented in the Americas, especially in the southeastern USA, and Australia (Kottwitz et al., 2014; Walden et al. 2020) (Table 1).

Spain is considered an endemic area, with recent studies reporting the presence of adult *A. cantonensis* specimens in synanthropic rodents (*R. rattus*, *R. norvegicus*, and *Mus musculus*) from Tenerife (Canary Island) and Valencia (Foronda et al., 2010; Martín-Alonso et al., 2011; Galán-Puchades et al., 2022, 2024). Additionally, larval stages have been found in hedgehogs in Mallorca (Balears Island) as accidental hosts (Paredes-Esquivel et al., 2019), in lizards as paratenic hosts from the Canary Islands (Anettová et al., 2022), and in snails as intermediate hosts from the Canary Islands (Martín-Alonso et al., 2011; Martin-Carrillo et al., 2023), Mallorca (Jaume-Ramis et al., 2023) and Valencia (Fuentes et al., 2024). The first European clinical case in humans involved a Cuban tourist in Catalonia, although this case was considered imported because he had consumed undercooked prawns (Valerio Sallent et al., 2021).

In this study, we described the lethal infection of *A. cantonensis* in three lemurs: two red-fronted brown lemurs (*Eulemur rufus*) and a ring-tailed lemur (*Lemur catta*) kept in captivity in a zoo in eastern Spain. These cases represent the first known reported fatalities due to angiostrongylosis in NHPs in Europe and the first known instances of this parasitic infection in both species worldwide.

2. Materials and methods

2.1. Case selection, clinical data, and pathology

Between December 2020 and February 2022, three cases of fatal meningitis occurred at the Bioparc zoo in Valencia, Spain (39°28′41″N, 0°24′27″W). The first two cases involved red-fronted lemurs, while the third was a ring-tailed lemur (Table 2). The first two lemurs were born at the zoo, while the ring-tailed lemur was donated by a center from the United Kingdom (UK) in May 2007. The first case died after 18 days of exhibiting neurological symptoms. The second and third cases were humanely euthanized due to severe paralysis and uncoordination, respectively.

All the individuals shared the same enclosure at the zoo, called ‘Madagascaŕ. In it, animals move freely from the ground to the trees. They are usually fed with fruits, vegetables, and feed. Pest control is carried out regularly throughout the premises by a private company. It focuses primarily on rodents and insects, especially cockroaches and mosquitoes.

2.2. Laboratory analyses

A complete necropsy was performed for each animal and samples from lung, heart, trachea, esophagus, stomach, intestine, pancreas, liver, spleen, kidney, adrenal gland, lymph nodes, and brain were taken. The spinal cord was obtained from one lemur. They were fixed in 10% buffered formalin, embedded in paraffin, processed for histological examination, and stained with H&E.

The nervous systems were systematically examined under a stereomicroscope (SMZ-171-TLED). Nematodes found were collected and preserved in 70% ethanol until later identification.

Total DNA was extracted from formalin-fixed paraffin-embedded bone marrow tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany)following the manufacturer’s protocols. Extracted DNA samples were kept at −80 °C until further analysis.

For the initial PCR amplification, universal primers for metastrongylid nematodes targeting the entire internal transcribed spacer 1 (ITS1) region were utilized as previously described (Izquierdo-Rodriguez et al., 2023). Subsequently, a second PCR was performed employing species-specific primers designed for *A. cantonensis*/*Angiostrongylus mackerrasae*, targeting the ITS1 region: ITS1_Canto_F3: AACAACTAGCATCATCTACGTC and ITS1_Canto_R1: CATCCTGTGTATCTCGTTCC; these were used as described previously (Izquierdo-Rodriguez et al., 2023; Qvarnstrom et al., 2010).

The partial sequence of the cytochrome *c* oxidase subunit 1 gene (COI) was amplified using the primers LCO1490: GGTCAACAAATCATAAAGATATTGG and HCO2198: TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994), following the PCR conditions described by Červená et al. (2019).

Angiostrongylus cantonensis genomic DNA and DNA-free distilled water were included as positive and negative controls, respectively. Amplification products were visualized on a 2% agarose gel electrophoresis in 1 × Tris-Borate-EDTA buffer and visualized using ultraviolet light after staining with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Sungnum, Korea).

The PCR products were purified using the QIAquick® PCR & Gel Cleanup Kit (Qiagen, Hilden, Germany) and sequenced by Stab Vida (Caparica, Portugal). Sequences were obtained from both strands, and a consensus sequence was generated utilizing the BioEdit Sequence Alignment Editor (version 7.2.5, Carlsbad, CA, USA). Species-level identity was determined with a >99% identity score threshold using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Mega-BLASTn option). Nucleotide sequences generated during this study were deposited in the DNA Data Bank of Japan (DDBJ: https://www.ddbj.nig.ac.jp/index-e.html).

2.3. Phylogenetic analyses

Phylogenetic analyses included five *Angiostrongylus* spp. (23 ITS1 sequences) and *Crenosoma striatum* (KP941434) as an outgroup. Maximum Likelihood (ML) reconstructions used the TPM2+F+I model (IQ-TREE) with Akaike’s criterion (Trifinopoulos et al., 2016), and tree robustness was assessed via SH-aLRT (Shimodaira-Hasegawa-like approximate Likelihood Ratio Test) and 5000 bootstrap resamplings.

Five *Angiostrongylus* spp. (18 sequences) were analyzed using the TIM3+F+I model (IQ-TREE) for COI. The same ML criteria and robustness tests were applied. Ambiguous positions were removed (pairwise deletion), yielding 685 positions in the final dataset.

Finally, the trees were visualized using FigTree v1.4.2 (https://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Pathological findings

Upon gross examination, all cases (3/3) exhibited diffuse moderate congestion of the meninges in the brain and cerebellum. In one case, multifocal irregular red areas measuring 3–4 mm were primarily localized in the cerebellum and brainstem (Fig. 1A). Several nematode fragments were found beneath the meninges and around blood vessels from cases 1 and 3 (Fig. 1B). It was not possible to identify the nematodes under the microscope due to their deficient conservation.

Histologically, the most affected areas of the brain were the meninges (3/3), corpus striatum (3/3), cerebellum (3/3), ventricular system (3/3), brainstem (2/3), and cerebral colliculus (1/2). The abundance of the parasites in different regions was highly variable, and they were sometimes associated or not with

Table 1
Historical cases of angiostrongylosis in non-human primates worldwide.

Author	Geographical location	Species	Age	Sex	Death cause	Location of necropsy	Clinical findings	Days of clinical signs	Anthelmintic Treatment	Pathological findings
Kodama et al. (1981)	Japan	Two *Macaca fascicularis*	NA	M F	Euthanasia	NA	Weakness, anorexia, paralysis of extremities, dysstasia	NA	NA	Eosinophilic meningitis, necrosis
Carlisle et al. (1998)	Australia	Four *Sanguinus oedipus oedipus* One *Sanguinus imperator subgrisescens*	3–5 6	3M, 1F M	Natural	NA	Depression, anorexia, paralysis	5 d–18 m	None	Eosinophilic meningitis
Gardiner et al. (1990)	Louisiana	*Abouatta caraya*	3	M	Natural	Audubon Park and Zoo logical Garden, New Orleans	Lethargy, ataxia	20	Sulfadimeth azone (5 mg/10 lb) and antihistamines	Meningoencephalitis
Kim et al. (2002)	Louisiana	*Varecia rubra*	NA	NA	Euthanasia	Zoo of Acadiana	Hyperpastic limbs	NA	None	Cerebral meninges multifocally hemorrhagic
Duffy et al., (2004)	Florida	*Hylobates lar*	49	M	Euthanasia	Miami Metrozoo	Quadriparesis, depression	2	None	Necrosis and inflammation in the dorsal gray columns throughout the cervical spinal cord
Emerson et al. (2013)	Florida	*Pongo pygmaeus*	6	F	Euthanasia	Private owner	Weakness of the left arm, mild fever, hiccup	19	Anti-inflammatory, antibiotic and fenbendazole	Meningoencephalitis
Kottwitz et al. (2014)	Alabama	*Saguinus geoffroyi*	3 4	M F	Natural Natural	Alabama zoo	Found dead	NA 7	NA	NA
Edwards et al. (2020)	Texas	*Callimico goeldii* *Saguinus oedipus* *Saguinus bicolor*	13 18 8	M M M	Natural Euthanasia Euthanasia	Texas zoo NA NA	Lethargy with weakness, diarrhea, ataxia, seizures Ataxia Seizures	2 7 3	NA	Meningoencephalitis
Walden et al. (2020)	Florida	*Cebus capucinus* *Varecia rubra*	17 NA	NA NA	Recovered	NA	Lethargy, hind end paresis		Fenbendazole (7 mg/kg PO q24 h × 14 d)	NA
Rizor et al. (2022)	Louisiana	*Varecia rubra*	9	M	Euthanasia	NA	hind limb paresis and a right head tilt	8	NA	Meningoencephalitis
Rivory et al. (2023)	Australia	*Saimiri boliviensis boliviensis*	11	M	Euthanasia	NA	NA	NA	NA	NA

M, male; F, female; NA, Not available.

Table 2
Medical history of three lemurs with angiostrongylosis from Valencia.

Case no.	Species	Age (years)	Sex	Cause of death	Clinical findings	Days of clinical signs	Anthelmintic treatment	Date of death
1	*Eulemur rufus*	8	Female	Natural	Right anterior lameness, apathy, posterior paralysis	18	None	2020 Dec 8
2	*Eulemur rufus*	6	Male	Euthanasia	Uncoordination, total posterior paralysis	13	Albendazole on day 6 (20 mg/kg/day, 5 consecutive days)	2020 Dec 22
3	*Lemur catta*	28	Male	Euthanasia	Uncoordination, falls onto its left site	14	None	2022 Feb 16

hemorrhages, necrosis, and inflammation (Fig. 1C–E). Hemorrhage, necrosis, and edema were frequently observed. When inflammation was present, it was mainly characterized by eosinophils, lymphocytes, plasma cells, macrophages and, rarely, giant cells (Fig. 1F–G). Perivascular cuffing occasionally occurred, with an expansion of Virchow-Robins space (Fig. 1H).

In the cervical spinal cord, parasites were observed in the leptomeninges (Fig. 1I). The histological findings are summarized in Table 3.

3.2. Parasitological findings

Microscopic examination of H&E stained sections from the three animals revealed larvae ranging from 150 to 200 µm in diameter. The larvae exhibited a smooth eosinophilic and thin cuticle, polymyaria-coelomiaria musculature, basophilic accessory hypodermic cords, a pseudocoelom, a digestive tract composed of a few multinucleated cells, and a genital canal (Fig. 1E). These features were compatible with immature nematodes of the superfamily Metastrongyloidea. Based on a combination of morphological characteristics, host species, and anatomical location, we suspected the species may be *A. cantonensis*.

3.3. Molecular analysis

PCR testing confirmed that three NHP samples were positive, however, only two displayed strong electrophoresis bands suitable for sequencing. After purification, both samples were successfully sequenced, identifying the species *A. cantonensis* with a nucleotide identity of 99.6–100% compared with the reference ITS1 sequences (*A. cantonensis*: **OR119902** and **OR119903**) and COI sequences (*A. cantonensis*: **MK570632** and **OR048341**) available in the GenBank database. Sequences have been deposited in the DDBJ database under accession numbers ITS1: **LC819639-40** and COI: **LC863979-80**. Phylogenetic ITS1 analysis showed clustering with *A. cantonensis*/*A. mackerrasae* sequences from Spain, the United States, and Australia (Fig. 2A). This distinction was further clarified through COI gene analysis, which separated both species, confirming the identity of our samples (Fig. 2B).

4. Discussion

To the authors' knowledge, this is the first study describing mortality from *A. cantonensis* infection in NHPs in Europe. We believe it is also the is the first known instance of neuroangiostrongylosis affecting *E. rufus* and *L. catta* worldwide, broadening the host range of this zoonotic parasite. Both species, as well as the rest of those belonging to the family Lemuridae, are among the most threatened group of mammals on earth. This discovery highlights the urgent need to include these pathogens in differential diagnoses and to enhance monitoring programs to protect these vulnerable species. Both NHPs are native to Madagascar, the last species being classified as threatened and considered the emblem of the Bioparc zoo.

The three cases presented here were fatal, with the animals exhibiting neurological signs for several days, associated with migration of the larvae and the immune response to dead nematodes (da Silva and Morassutti, 2021). In one case (no. 2), anthelminthic treatment with albendazole was administrated when the clinical signs started, but no positive response was observed. Although previous infected NHPs, such as capuchin monkeys and lemurs, have recovered after treatment with fenbendazole (7 mg/kg per os every 24 h × 14 days) (Walden et al. 2020), most cases reported in NHPs resulted in death or required euthanasia. This may indicate more severe immune reaction or treatment challenges in these species than in humans, in whom the disease is often self-limiting, manifesting as acute headache and malaise for several weeks. Additionally, underdiagnosis or late diagnosis must be considered (Duffy et al., 2004). In these valuable animals, an early diagnosis employing advanced tools such as real-time PCR on cerebrospinal fluid becomes fundamental for preventing the disease. In human patients with eosinophilic meningitis, this technique detected larval DNA in 22 of 33 cerebrospinal fluid samples (Qvarnstrom et al., 2016).

Histopathological examinations of the three lemurs revealed the presence of immature adult stages of the parasite in nervous system tissue, although adult specimens have been described in tamarins (*Saguinus* spp.) (Carlisle et al., 1998; Edwards et al., 2020). These parasites naturally migrate to the lungs in rodent hosts but generally cease migration in the nervous system of accidental hosts. However, migration to the lungs has been shown by Edwards et al. (2020) in a pied tamarin (*Saguinus bicolor*). Although it was impossible to identify the entire specimens under the microscope, the histological description suggests that at least 3 weeks had elapsed since L3s were ingested. Post-mortem analysis revealed no significant lesions apart from congestion in all three cases and multiple areas of hemorrhage in case 3. This aligns with the absence of macroscopic brain and spinal cord lesions in a red-ruffed lemur (*Varecia rubra*) (Rizor et al. 2022). Previous reports have described the locations of nematodes in the brain, brainstem, cerebellum, and spinal cord in red-ruffed lemurs (Edwards et al., 2020; Rizor et al., 2022). In the present study, however, nematode larvae were also free in the brain ventricles in all three cases. There could be several mechanisms by which parasites can pass from the meninges into the ventricles. One possible mechanism is the parasite migrating through the tissues by perforating the meninges into the ventricles. Alternatively, haematogenous spread could allow parasites to enter the bloodstream and be transported to the brain ventricles, where they could cross the blood–brain barrier into the cerebrospinal fluid. In this study, the parasites were localized in the cervical region of the spinal cord in case 2; previous studies had described their presence in the thoracic and lumbar regions of captive callitrichids (Edwards et al., 2020).

The main histopathological findings included necrosis and hemorrhage, with areas of mild to severe inflammation comprising eosinophils, Gitter cells, lymphocytes, and plasma cells around

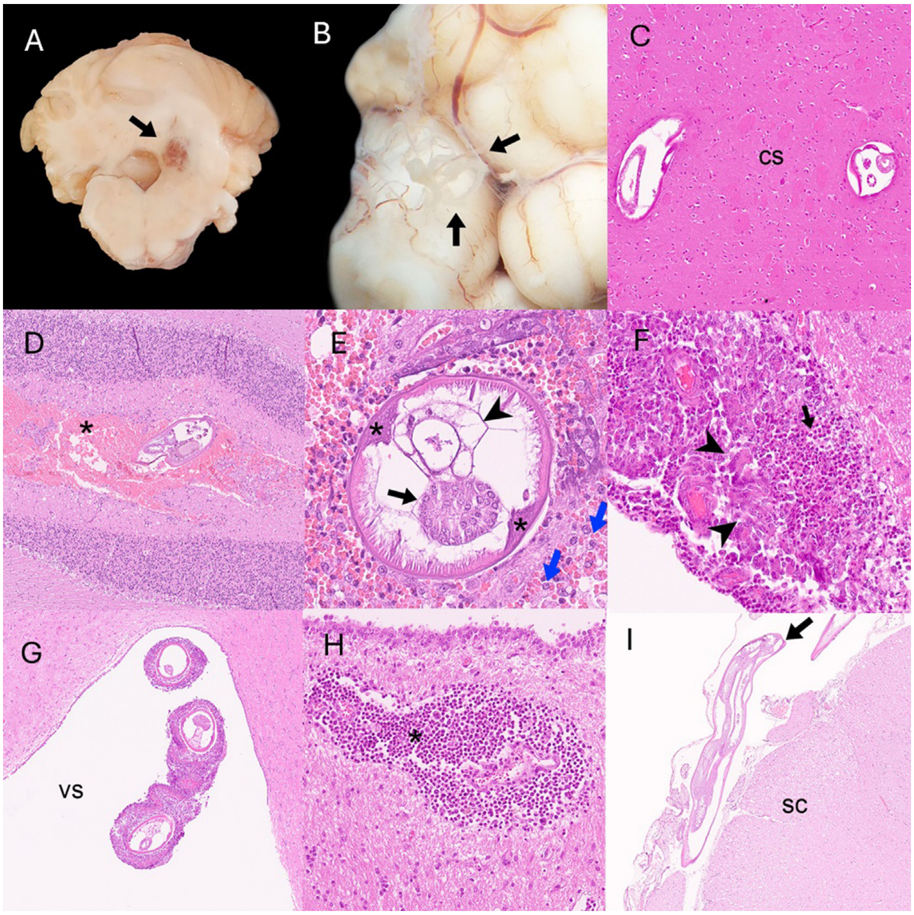

Fig. 1. Gross and histological findings associated with *Angiostrongylus cantonensis* in lemurs from Valencia, Spain. (A) Irregular area of necrosis and hemorrhage of 3–4 mm located between the cerebellum and brainstem (arrow). Case 3. (B) Nematode larvae localized in the meninges of the cerebellum (arrows). Case 3. (C) Corpus striatum. Two nematodes in cross sections with no pathological reaction (hemorrhage, necrosis, and/or inflammation). Case 2. H&E. (D) Cerebellum. Severe hemorrhage (asterisk) with migration of nematode. Case 1. H&E. (E) Cerebral colliculus. Cross-section of a nematode showing cuticle, polymyaria-coelomiaria musculature, basophilic accessory hypodermic cords (asterisks), pseudocoelom, intestine (arrowhead), and the genital canal (arrow). Gitter cells (arrows on right) and hemorrhage associated with the nematode. Case 1. H&E. (F) Meninges. Foci of inflammation with eosinophils (arrow), multinucleated giant cells (arrowheads), neutrophils, and fewer lymphocytes and plasma cells. Case 3. H&E. (G) Ventricular system. Three cross-sections of nematodes with surrounding eosinophilic inflammatory reaction. Case 2. H&E. (H) Ventricular system. Perivascular cuffing (asterisk) with expansion of the Virchow-Robins space by epithelioid macrophages, lymphocytes, and plasma cells. Case 1. H&E. (I) Spinal cord. Longitudinal section of the nematode in meninges without pathological lesions. Case 2. H&E. CS, corpus striatum; VS, ventricular system; SC, spinal cord.

the parasites, findings similar to those described in the Bolivian squirrel monkey, and red ruffed lemur in other studies on other species (Edwards et al., 2020; Rizor et al., 2022; Rivory et al., 2023). Among the three lemurs, and even within the same animal, histological lesions varied. Some areas exhibited parasites surrounded exclusively by hemorrhage and rarefaction of the neuropil, indicating an acute course of infection without inflammation. Other areas showed only variable inflammation (eosinophils, macrophages, lymphocytes, and plasma cells), suggesting a subacute course. Remarkably, the absence of parasite-associated pathological changes (necrosis, hemorrhage, and inflammation) was also observed, which has not been previously described. This variation may be related to the number of ingested larvae, the time passed since ingestion, and whether it was a primary or re-infection. Considering that a single snail may harbour from a few to thousands of infective larvae, the severity of the lesions can vary significantly among susceptible hosts.

The rat lungworm is considered an emerging zoonotic parasite in Europe, having been found parasitizing both intermediate and definitive hosts from different countries over the last decade. Concretely, Galán-Puchades et al. (2022) confirmed its presence in rats from Valencia, marking the first known finding of this species in the Iberian Peninsula. In this study, positive rats were found in the same district where the zoo is located. Recent research has also isolated DNA of the parasite from three terrestrial snail species as intermediate hosts (Fuentes et al., 2024) in this district, confirming that the worm's life cycle can be completed in the study area. Besides, zookeepers observed lemurs consuming snails from the ground, a behaviour previously confirmed in other NHPs (Walden et al., 2020).

According to the ITS1 phylogenetic analyses, our sequences clustered with those recently detected in the Canary Islands and the Balearic Islands (Spain), and those from the United States. These sequences grouped alongside those of *A. mackerrasae*. However, the latter species must be ruled out, as it has not been detected outside Australia to date (Morgan et al., 2021). On the other hand, our COI sequences clustered with those from Hawaii and French Polynesia, forming a distinct group separate from another cluster that included sequences from Australia, the continental USA, and Spain (Canary Islands). However, this grouping was supported by a limited bootstrap value of 75%, confirming the low genetic diversity trend among invasive *A. cantonensis* isolates, as reported by other authors (Dusitsittipon et al., 2018; Červená et al., 2019).

The rapid spread of *A. cantonensis* to new territories in recent decades is alarming (Cowie et al., 2022). The Port of Valencia, the fourth largest in Europe (https://www.valenciaport.com/en/), can be a major gateway due to its strong trade connections with the

Table 3
Pathological features in the three lemurs with angiostrongylosis from Valencia, Spain.

	Brain							Spinal cord			Other lesions
Case no.	Parasite location	Meningitis	Hemorrhage	Necrosis	Gitter cells	Eosinophilic inflammation	Perivascular cuffing	Parasite location	Meningitis	Axonal degeneration	
1	Meninges, corpus striatum, cerebellum	[a]	[b]	[b]	[b]	[a]	[a]	NS	NS	NS	Moderate, pulmonary congestion; hepatic glycogenosis
2	Meninges, corpus striatum, cerebellum, brainstem, cerebral colliculus	[a]	[b]	[b]	[a]	–	–	Meninges	[a]	[a]	Hepatic glycogenosis
3	Meninges, corpus striatum, cerebellum, brainstem	[b]	[c]	[c]	[b]	[a]	[a]	NS	NS	NS	Moderate, pulmonary, and hepatic congestion; hepatic glycogenosis

NS, not sampled.
[a] Mild.
[b] Moderate.
[c] Severe.

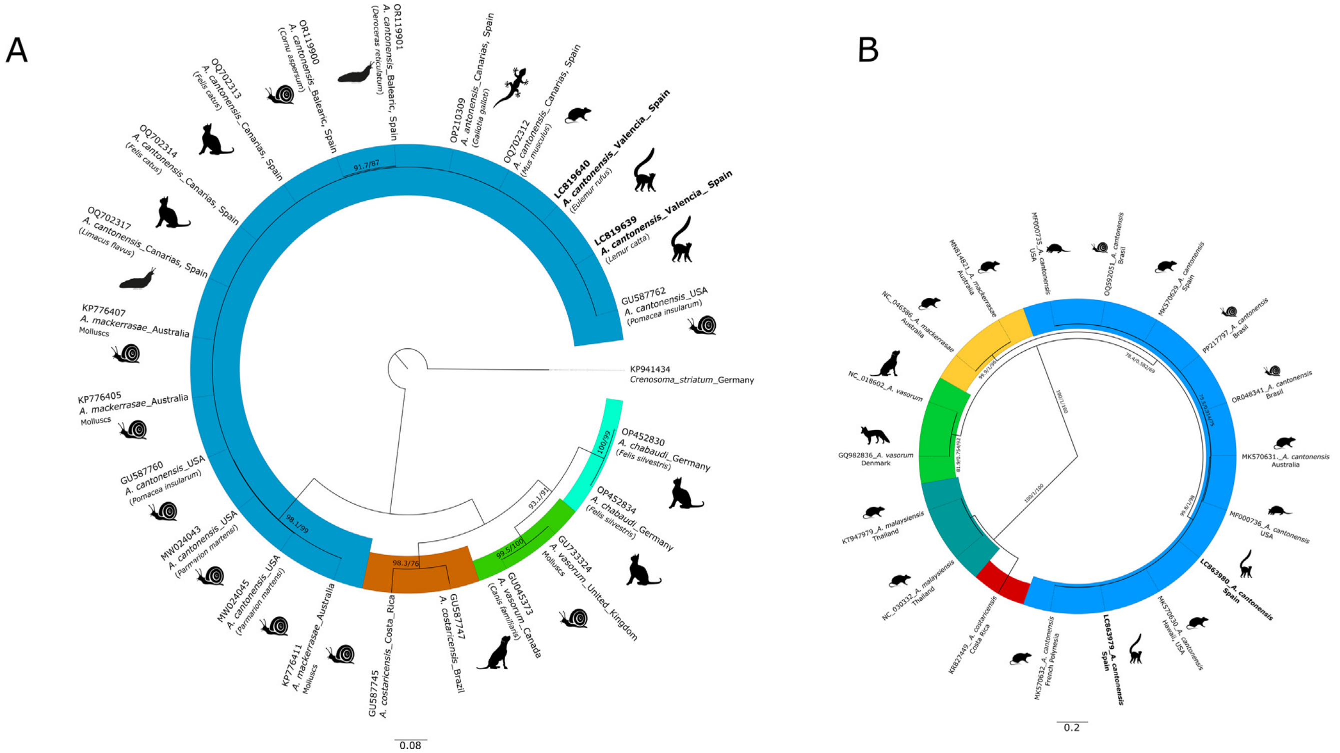

Fig. 2. A circular phylogenetic tree of *Angiostrongylus* spp. (A) Phylogenetic tree based on the 23 internal transcribed spacer 1 (ITS1) gene nucleotide sequences, inferred using the Maximum Likelihood (ML) method and rooted with *Cresomia striatum* ([database? Accession number KP941434) as the outgroup. (B) Phylogenetic tree based on 18 cytochrome *c* oxidase subunit I (COI) gene nucleotide sequences, also inferred using the ML method. Bootstrap values >75% are indicated at specific branch nodes. The scale bar represents the average number of substitutions per site. Sequences obtained in this study are shown in boldface.

USA and China. Frequent and direct maritime traffic between the Spanish islands, the port of Valencia, and regions where this pathogen is endemic have likely facilitated its introduction via cargo ships or the unintentional importation of infected rats and gastropods (González and Ruiz de Ybáñez, 2022; Galán-Puchades et al., 2024). Furthermore, introducing mollusks and other invertebrates into local ecosystems, often linked to cultural consumption practices, could be crucial in expanding the parasite's life cycle in the Iberian Peninsula.

Europe was considered a non-endemic region until 2018 when the nematode was detected infecting two North African hedgehogs (*Atelerix algirus*) on the Spanish island of Mallorca (Paredes-Esquivel et al., 2019). Recently, human cases have increased among travelers returning from endemic countries (Valerio Sallent et al., 2021). Risk factors include the consumption of raw or undercooked snails, which are part of traditional dishes in some European countries such as Spain or France. Furthermore, recent studies have also shown the capacity of larvae to overwinter inside the snails, highlighting the parasite's survival potential (Anettová et al., 2023). It has been also suggested that NAS cases in humans may result from accidentally ingesting infected slime from snails or slugs on vegetables, which can be prevented by thoroughly washing and rinsing produce, especially green leafy vegetables (e.g., lettuce) (Tsai et al., 2004).

Commensal rodents in zoos have been documented as hosts for various zoonotic pathogens. In Portugal, five species of zoonotic endoparasites were identified in commensal rodents at Lisbon Zoo: *Hymenolepis diminuta* and *Cysticercus fasciolaris* (Cestoda), *Syphacia obvelata* and *Calodium hepaticum* (syn. *Capillaria hepatica*) (Nematoda), and *Cryptosporidium* sp. (Protozoa) (Crespo et al., 2013). In the Czech Republic, antibodies against *Coxiella burnetii*, *Francisella tularensis*, and *Borrelia burgdorferi* sensu lato were detected in wild rodents collected at Brno Zoo (Pittermannová et al., 2021). In France, pest rodents were identified as potential sources of contamination with *Lawsonia intracellularis*, isolated from several pileated gibbons (*Hylobates pileatus*) exhibiting diarrhea at Mulhouse Zoo (François-Brazier et al., 2021). In Spain, sympatric brown rats (*Rattus norvegicus*) from the Cordoba Zoo exhibited a prevalence and seroprevalence of *Leptospira* spp. of 18.2% and 41.9%, respectively. This occurred precisely where an outbreak of acute mortality was observed in endangered Barbary macaques (*Macaca sylvanus*) kept in captivity (Beato-Benítez et al., 2024). This situation highlights the importance of strengthening control measures for rodents inhabiting zoos, which provide ample resources (e.g., abundant food, water, shelter, and ideal temperatures) for their proliferation. Given the exceptional ability of certain animals, particularly primates, to access products deployed for pest management, avoiding rodenticides or bait stations within their enclosures is strongly recommended. Instead, perimeter control should be reinforced throughout the zoo premises and the facilities, employing snap traps. Additionally, routine environmental control measures, including eliminating burrows and removing excess food, should also be implemented.

The nematode *A. cantonensis* is emerging in Europe and appears well-established in Valencia, representing the only reported location in mainland Spain. Given this parasite can infect a wide variety of mammals including humans, medical and veterinary community professionals must implement surveillance programs for this zoonotic pathogen. Early detection of the infection seems challenging, and treatment is not always effective; therefore, emphasis must be placed on prevention. It is crucial to implement the same recommended measures in NHPs as for humans, such as avoiding the consumption of inadequately washed vegetables and raw snails. Additionally, more efficient rodent control and monitoring of terrestrial snails and slugs should be implemented in urban and anthropized areas to mitigate the risk of transmission.

CRediT authorship contribution statement

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