



Pneumonia by *Aelurostrongylus abstrusus* in a *Leopardus tigrinus*: Case study

Neumonía por Aelurostrongylus abstrusus en un Leopardus tigrinus: Estudio de caso

Pneumonia por Aelurostrongylus abstrusus em um Leopardus tigrinus: Estudo de caso

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Abstract

Anamnesis: A polytraumatized wild oncilla (*Leopardus tigrinus*) was admitted to the Unidad de Rehabilitación y Rescate de Animales Silvestres (URRAS) veterinary clinic of the Universidad Nacional de Colombia. Despite the medical efforts, the animal died six hours later. **Clinical and laboratory findings:** Necropsy examination revealed multiple white nodules of about 2 mm in diameter distributed on the visceral surface of lung caudal lobes. Histopathology revealed lymphoplasmacytic interstitial pneumonia with multiple coiled larvae of metastrongyloid nematodes in alveoli and bronchioles. First-stage *Aelurostrongylus abstrusus* nematode was identified in a fecal sample using the Ritchie copromicroscopic technique. **Conclusion:** This study reports an incidental severe pulmonary parasitism caused by *A. abstrusus* in a wild Oncilla (*L. tigrinus*). This is the first report of *A. abstrusus* infection associated with pathological lesions in a *L. tigrinus* from Colombia.

Keywords: *Aelurostrongylus abstrusus*; *Leopardus tigrinus*; little spotted cat; little tiger cat; lung; lungworm; lung parasitism; multiparasitism; nematode; oncilla; parasites; pneumonia; wildlife.

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Resumen

Anamnesis: Un tigrillo silvestre (*Leopardus tigrinus*) politraumatizado fue ingresado en la clínica veterinaria Unidad de Rehabilitación y Rescate de Animales Silvestres (URRAS) de la Universidad Nacional de Colombia, donde posteriormente falleció. **Hallazgos clínicos y de laboratorio:** El examen de necropsia reveló múltiples nódulos blancos de aproximadamente 2 mm de diámetro, distribuidos en la superficie visceral de los lóbulos caudales del pulmón. La histopatología reveló neumonía intersticial linfoplasmocítica, con múltiples larvas enrolladas de nematodos metastrongiloides en alvéolos y bronquiolos. En la muestra fecal postmortem se identificó el nematodo *Aelurostrongylus abstrusus* en primera etapa por la técnica copromicroscópica de Ritchie. **Conclusión:** Este estudio reporta un parasitismo pulmonar severo incidental causado por *A. abstrusus* en un tigrillo silvestre (*L. tigrinus*). Este es el primer reporte de infección por *A. abstrusus* asociada con lesiones patológicas en un *L. tigrinus* en Colombia.

Palabras clave: *Aelurostrongylus abstrusus*; fauna silvestre; leopardo tigre; *Leopardus tigrinus*; multiparasitismo; nematodo; neumonía; oncilla; parasitismo pulmonar; parásitos; pulmón; tigrillo.

Resumo

Anamnese: Um Maracajá (*Leopardus tigrinus*) selvagem de vida livre foi internado na clínica veterinária Unidad de Rehabilitación y Rescate de Animales Silvestres (URRAS) da Universidad Nacional de Colombia devido a politraumatismo e o animal morreu. **Achados clínicos e laboratoriais:** O exame de necropsia revelou múltiplos nódulos brancos com cerca de 2 mm de diâmetro distribuídos na superfície visceral do pulmão dos lobos caudais. A histopatologia revelou pneumonia intersticial linfoplasmocitária com múltiplas larvas enroladas de nematóides metastrongilóides em alvéolos e bronquíolos. A amostra fecal post mortem para a técnica copromicroscópica de Ritchie permitiu a identificação do nematóide *Aelurostrongylus abstrusus* de primeiro estágio. **Conclusão:** Este estudo relata um grave parasitismo pulmonar incidental causado por *A. abstrusus* em um Maracajá selvagem de vida livre (*L. tigrinus*) da Colômbia. Este é o primeiro relato de infecção por *A. abstrusus* associada a lesões patológicas em *L. tigrinus* da Colômbia.

Palavras-chave: *Aelurostrongylus abstrusus*; fauna silvestre; gato do mato; gato-lagartixeiro; *Leopardus tigrinus*; maracajá; multiparasitismo nematóide; pneumonia; parasitismo pulmonar; parasito; pintadinho; pulmão; oncilla.

Introduction

Aelurostrongylus abstrusus is a nematode of the family Angiostrongylidae, within the Strongylida order. *A. abstrusus* is the most important nematode of the respiratory system in domestic cats (*Felis silvestris catus*) and is distributed worldwide (Traversa and Di Cesare, 2016). *A. abstrusus* cause occasional infections in several wildcats (*Felis silvestris silvestris*) from Europe (Traversa and Di Cesare, 2016), and also in serval cats (*Leptailurus serval*) and lions (*Panthera leo*) in Africa (Di Cesare *et al.*, 2016). Infection by *A. abstrusus* has also been reported in South American *Leopardus tigrinus*, *L. colocolo* and in *Puma yagouaroundi* (Penagos-Tabares *et al.*, 2018).

A. abstrusus is characterized by having an indirect life cycle with gastropods acting as intermediate hosts (Anderson, 2000); the L1 requires hosts such as land snails (*Helix* spp. and *Lissachatina fulica*), land slugs (*Deroceras* spp. or *Arion* spp. and *Arion lusitanicus*) (Jezewski *et al.*, 2013; Valente *et al.*, 2017) or a paratenic host such as rodents, reptiles, birds or frogs (Elsheikha *et al.*, 2016). The *A. abstrusus* L3 is the infective larval stage for the definitive host (Anderson, 2000). The L1 penetrates snails and slugs, where they develop to L3. Once ingested, the infective L3 penetrates the intestinal mucosa of the definitive host and reaches to the lungs via lymphatics (Elsheikha *et al.*, 2016).

Infection by *A. abstrusus* ranges from subclinical to severe clinical manifestations that may include abdominal breathing with an open mouth, intense coughing, sneezing, mucopurulent discharge, dyspnea, and hydrothorax. In more severe infections the animal may develop interstitial pneumonia (Traversa *et al.*, 2008). Secondary bacterial infections can occur in severe cases, leading to death (Salamanca *et al.*, 2003). Diagnosis is based on direct observation of the parasite in fecal samples or airway wash examination (Traversa and Di Cesare, 2016).

A. abstrusus was previously reported in

domestic cats in Colombia (Penagos-Tabares *et al.*, 2018), and more recently using the Baermann method in cats from Antioquia, Colombia (López-Osorio *et al.*, 2021). The parasite was observed in a case using the Ritchie technique (Salamanca *et al.*, 2003). To the best of our knowledge, the present report is the first describing pathological lesions caused by *A. abstrusus* infection in a wild *L. tigrinus* from Colombia. *L. tigrinus* is currently considered a vulnerable species by the International Union for the Conservation of Nature (IUCN) (<https://www.iucnredlist.org/species/54012637/50653881>, accessed on November 5, 2021).

Case presentation

Anamnesis

A wild adult *L. tigrinus* from the Colombian Andes (Chipaqué municipality, Cundinamarca province -North Latitude 4.4425, West Longitude 74.0414) was admitted with severe polytraumatism to the “Unidad de Rehabilitación y Rescate de Animales Silvestres (URRAS) of the Universidad Nacional de Colombia”. The trauma was related to a car incident on the road.

Clinical findings and diagnostic aids used

Clinical examination revealed a deep wound with partial exposure of the omentum in the ventral region of the abdomen, pneumothorax, and complete fracture of the right ilium. The feline was subjected to surgical procedures to correct the bone fracture and omentum exposure. Despite the medical efforts, the animal died six hours later. The necropsy showed poor body condition, pale mucous membranes and multiple hematomas on the ventral abdomen. The 11th and 12th ribs on the right side of the rib cage were fractured, as well as the right ilium. The lung was partially collapsed, had diffuse congestion and edema, and 2 mm white nodules randomly distributed on the visceral pleura of caudal lobes and parietal pleura of the chest wall were observed (Fig. 1). Lung tissue samples were collected and fixed in 10% neutral buffered formalin, routinely processed and stained with hematoxylin and eosin (H&E).

Histopathology revealed multiple coiled metastrongyloid nematode larvae in the sagittal section (80-150 μm) and cross-section (12-16 μm) within the lumen of bronchi, bronchiole and alveoli of the lung (Fig. 2). The parasites were associated with severe multifocal lymphocytic and plasmacytic interstitial

pneumonia. Some macrophages and neutrophils were also present. Moderate amount of mucus, cell debris and some desquamated epithelial cells were present in the lumen of the bronchi. In addition, alveolar atelectasis, moderate multifocal hemorrhage, edema and diffuse congestion were observed.

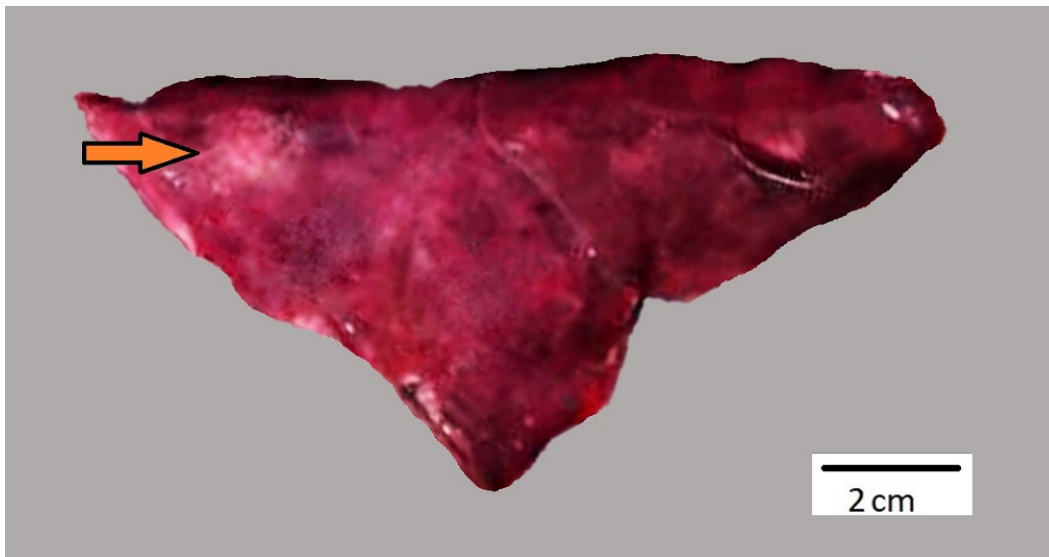


Figure 1. Lung of a *Leopardus tigrinus* naturally infected with *Aelurostrongylus abstrusus*. Multiple white nodules of approximately 2 mm in diameter were found near the caudal region (arrow) of the lung. Moderate diffuse pulmonary edema and congestion was also present. Bar, 2 cm.

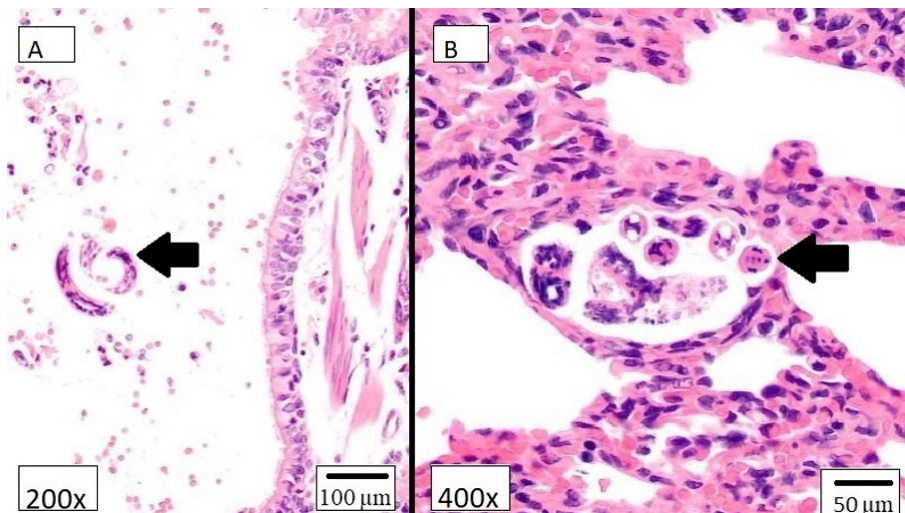


Figure 2. Severe verminous interstitial pneumonia in a *Leopardus tigrinus* caused by *Aelurostrongylus abstrusus*. The left panel shows coiled nematode larvae (arrow) in the lumen of the bronchi. The right panel shows moderate interstitial pneumonia, characterized by a lymphoplasmacytic infiltrate associated with intralesional parasitic forms (the arrow indicates a transversal portion of the nematode). H&E stain.

Following the Ritchie technique, the first step was comminution of the fecal sample, continuing with a straining with two layers of gauze. Fecal sediment was added with 10 ml of 10% formalin, allowing five minutes for fixation of parasite structures and continuing with the addition of 3 ml ether to the formalinized solution. The specimen was then centrifuged at slow speed (1,500 rpm for two minutes). Ether, superficial debris and formalin were completely decanted using an applicator to free superficial debris from the centrifuge tubes. The last step consisted in coverslip preparations. The remaining sediment was mixed with the fluid and poured onto a slide. A small drop of 2% iodine solution was placed near the drop of the sediment and mixed. Finally, the coverslip was pushed into the drop (Ritchie, 1948). *A. abstrusus* (Railliet, 1898) L1 were identified in the sample (Fig. 3). *A. abstrusus* L1 in feces were 360-400 μm long (median range: 370 μm and 15-20 μm in diameter). *A. abstrusus* appeared as a short and thick larva with sub-terminal spine on its S-shape, with conical head and granular content. Ancylostomatidae eggs (*Ancylostoma* spp., *Uncinaria* spp.) and *Capillaria* spp. were also observed.



Figure 3. Morphological features of *Aelurostrongylus abstrusus* L1, measuring between 360-400 μm in length, detailing its tail and head. Bar, 10 μm .

Discussion

Although *A. abstrusus* is routinely found in domestic cats, its presence and potential pathological effects in wild cats is mostly unknown. In this report, the necropsy of an injured and finally dead *L. tigrinus* and the histopathological analysis of tissue samples revealed a number of lesions associated with severe *A. abstrusus* larvae infection. The intralesional parasitic larvae, observed in sagittal and cross-sections, caused a moderate interstitial pneumonia characterized by lymphocyte and plasmacyte infiltration. Parasitological studies confirmed the diagnosis. Since the animal was admitted to the clinic as a case of multiple traumas, which is also the most probable cause of death, the subclinical or clinical nature of *A. abstrusus* larvae infection remains unknown. Both pulmonary and gastrointestinal parasitosis could contribute to a poor body condition and weakening of the animal. Polytraumatism likely occurred when the animal and a vehicle collided on a roadway, worsening the health status and ending in the death of the animal. It is also possible that the anesthesia procedure conducted in the parasitized cat may have contributed to the deadly result. In this regard, previous studies report that anesthesia procedures were associated with death in domestic cats infected with *A. abstrusus* (Gerdin *et al.*, 2011).

The *A. abstrusus* infection caused a lymphoplasmacytic interstitial pneumonia, which has also been reported in infected domestic cats (Philbey *et al.*, 2014), where macrophages and eosinophils are associated with the parasites. Interstitial pneumonia has been reported mainly in subacute infections (Traversa *et al.*, 2008), while a previous study described interstitial pneumonia in experimentally infected domestic cats (Schnyder *et al.*, 2014). Tissue damages observed in *L. tigrinus* in the present case are very similar to those described in fatal natural or experimental cases in domestic cats (Schnyder *et al.*, 2014; Traversa *et al.*, 2014). Since all available reports of *A. abstrusus* infection have been documented in domestic cats, it was not possible to compare the pathological lesions of

L. tigrinus with other wild cats; therefore, this report constitutes the first case of *A. abstrusus* infection in a wild feline in Colombia.

Diagnosis of pulmonary parasitosis caused by *A. abstrusus* larvae was established and supported by the data collected from the Ritchie technique along with the morphological characterization of the parasitic larvae. The Ritchie technique is known for increasing the likelihood of finding ova, cyst, and larvae, particularly in those specimens where they are present in insufficient numbers (Manser *et al.*, 2016) and it is very useful in small size samples from wild animals with unknown parasitological records.

Molecular techniques such as PCR may be useful to confirm the identity of parasite species in animal tissues. Since extraction of DNA with suitable quality for PCR is not an easy procedure from formalin fixed and paraffin embedded tissues, recently, the morphological characterization of *A. abstrusus* and *Angiostrongylus chabaudi* and the location of adult nematodes was proposed as an alternative methodology to differentiate them (Wulcan *et al.*, 2020). In addition, Giannelli and collaborators summarized the main lesions observed in different feline metastrongyloid infections and reported that *A. abstrusus* is usually present in respiratory bronchioles and alveolar ducts while species of *Angiostrongylus* mainly affects the pulmonary arteries (Giannelli *et al.*, 2016). In the present case, no lesions were observed in blood vessels, whereas the presence of morulated eggs and larvae within the airways and alveolar ducts of the lung sections were indicative of *A. abstrusus* infection. Although the pathological lesions observed in *L. tigrinus* were mostly associated with *A. abstrusus*, it is important to note that the animal had a mixed parasitosis where *Ancylostoma* spp., *Uncinaria* spp., and *Capillaria* spp. may have also contributed to the poor body condition and was prone to traumatic coalition. In Brazil, up to 12 different parasite species in 14 fecal samples from *L. tigrinus* and a significant proportion of samples (35.7%) were reported positive to *A. abstrusus* (Kusma *et al.*, 2015).

Large scale epidemiologic studies are limited. Research in Italy found *A. abstrusus* in 1.82 (Site A) and 9.96% (Site B) of individual fecal samples of domestic cats (total sample size: 970; Di Cesare *et al.*, 2011). In a large European study of lungworms in domestic cats, 1,990 animals were sampled, from which 613 (30.8%) were positive for at least one parasite, while 210 (10.6%) were infected with lungworms, and *A. abstrusus* was the species most frequently detected (78.1%) (Giannelli *et al.*, 2017). Recently, an experimental semi-nested PCR in blood samples of 171 domestic cats in Chile found 19.9% molecular positivity (Barrios *et al.*, 2021). In Brazil, two cases of 81 cats (2.5%) were positive for metastrongyloid L1 at the Baermann's test, and one case was confirmed for *A. abstrusus* by a molecular test (Silva *et al.*, 2021). Recently, an epidemiological study reported, for the first time, the presence of *A. abstrusus*, *Troglostrongylus brevior*, *Crenosoma vulpis*, and *Angiostrongylus vasorum* in the invasive giant African snail (*L. fulica*) in several locations of Colombia. *A. abstrusus* was found in 9.2% of *L. fulica*, with all positives present in a single municipality (Penagos-Tabares *et al.*, 2019).

A. abstrusus has been reported in domestic cats in Colombia: A case in Quindío province from 121 samples processed (Echeverry *et al.*, 2012), a case from Caquetá province (Penagos-Tabares *et al.*, 2018), L1 was found in two cats from 473 sampled in Antioquia province using the Baermann method (López- Osorio *et al.*, 2021), and the parasite was observed in a case using the Ritchie technique in Bogotá (Salamanca *et al.*, 2003). The distribution of *A. abstrusus* in Colombia is unknown because the parasite is limited to case reports and few studies.

Currently, the impact of *A. abstrusus* in wild felids is unknown. Whether unnoticed relationships between wild and domestic cats may affect the health status of domestic cats is also unknown. The absence of epidemiological information on *A. abstrusus* in domestic and, probably, wild cats can be explained by at least two reasons: a predominance of subclinical infections, or underdiagnosed parasitosis (Traversa *et al.*, 2008).

Feline aelurostrongylosis is distributed worldwide and is often erroneously considered to be sporadic despite being a common infection (Di Cesare *et al.*, 2011).

The ‘dilution effect’ hypothesis suggests that the net effects of biodiversity (including host and non-host species) reduce the risk of certain diseases in ecological communities (Keesing *et al.*, 2006). There is evidence of this effect on parasites ecology (Civitello *et al.*, 2015). Predators can induce trait-mediated indirect effects (TMIEs) on parasites and vice versa, and these effects could have important implications for disease emergence and parasite regulation (Raffel *et al.*, 2008). Understanding the influence of predation on parasite transmission requires explicit examination of the host, parasite, and environmental conditions that influence parasite vulnerability (Orlowski *et al.*, 2015). The role of intermediate or paratenic host in the ecology of *A. abstrusus* is not fully understood. This knowledge is highly relevant for a mega-diverse country such as Colombia.

Reports of *A. abstrusus* infection in wild felids diagnosed either by examining fecal samples or histopathology are very limited. Being the first report of lungworms in a wild felid from Colombia, this study warns about lungworm infection of wild cats and its potential impact on animal health and wildlife conservation.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Necropsy, histopathology, literature review and manuscript writing: Diego F. Rincón-Alarcón. Histopathology, critical review and manuscript writing: Benjamín Doncel. Morphology characterization of nematode and eggs of other species, critical review and manuscript writing; Jesús A. Cortés-Vecino. All Authors revised the final version.

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