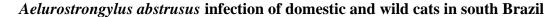
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Abstract: Aelurostrogylus abstrusus is a helminthic pathogen that infects the respiratory tract of felids after the ingestion of intermediate or paratenic hosts. The present paper assesses A. abstrusus infection in domestic and wild cats in south of Brazil. Fecal samples from 97 domestic cats and from seven wild cats (Puma concolor, Puma yagouaroundi and Leopardus tigrinus) were tested using sedimentation and flotation techniques. The prevalence of first-stage larvae was 2.1% (2/97) in domestic cats, and Puma yagouaroundi was the only wild cat that turned out to be positive for this lungworm. This is the first report of A. abstrusus in Puma yagouaroundi in Brazil. The identification of this parasite raises a red flag about the presence of infected intermediate and/or paratenic hosts and about the importance of this parasitic disease even in the absence of respiratory clinical signs. The analysis of fecal samples is then suggested for the detection of *A. abstrusus*.

Key words: Aerulostrongylus abstrusus - Cat - Wild cat - Coprological methods

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Introduction

Aelurostrogylus abstrusus (Railliet 1898) is a helminthic parasite of the respiratory tract of felids, having a complex life cycle and causing infection indirectly ingestion the of intermediate hosts infected by molluscs Deroceras, Arion, of the genera Agriolimax, Helicella, Limax and Theba or of paratenic hosts such as birds, rodents, amphibians, and lizards

carrying third-stage larvae (L3) (Nabais et al. 2013). After ingested by the definitive host, the larvae migrate through the gastrointestinal tract and bloodstream, lodge in the branches of pulmonary arteries, alveoli, alveolar ducts, and bronchioles (Ribeiro and Lima 2010), and undergo two molts before they become adults.

Thereafter, they reproduce, hatch in the alveoli and develop into first-stage larvae (L1), which are coughed up, swallowed, and eventually passed in the feces (Ellis et al. 2010). Clinical signs vary according to the severity of infection, to the age of the animal, to immune system involvement, parasite load, and to associations with other conditions. Lungworm infections in healthy individuals are subclinical and self-limiting, but in immunocompromised animals, clinical signs include labored breathing, sneezing, cough, mucopurulent nasal discharge, dyspnea, tachypnea, and eventually lead to death, as a result of oviposition in the host's bronchi, triggering an inflammatory response consequently, and, causing granulomatous with pneumonia hyperplasia and hypertrophy of the smooth muscles of the parenchyma and pulmonary arteries (Barutzi and Schaper 2012; Traversa and Di Cesare 2013). The diagnosis of A. abstrusus is based on the detection of larvae (L1) in fecal samples. The sedimentation method (Hoffman et al. 1934) is the gold standard for the diagnosis of this infection (Traversa et al. 2010).

Tracheal or bronchoalveolar lavage, albeit invasive, may be used, as well as thoracic radiographs (Lacorcia et al. 2009); serological tests, however, can show cross-reactivity between

endoparasite species (Traversa and Guglielmini 2008), whereas molecular methods have been found to have higher sensitivity and specificity (Traversa et al. 2008a). The aim of this paper is to assess the prevalence of *A. abstrusus* larvae in fecal samples from domestic and wild cats in southern Brazil.

Materials and methods

Fecal samples were collected from 97 stray cats captured from several neighborhoods in Lages (27° 49′ 0″ S and 50° 19′ 35″ W), in the mountain region of the state of Santa Catarina, southern Brazil. The cats were housed at the local Center for Zoonotic Disease Control. 24-hour stool samples were collected from the floor of individual stalls between May and November 2012.

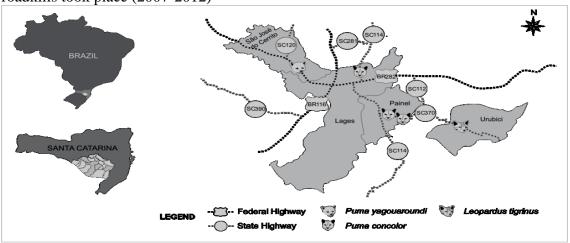
Seven dead adult wild cats, including *Puma concolor* (3), *Leopardus tigrinus* (3) and *Puma yagouaroundi* (1), which had been struck and killed on two highways (1 federal and 1 state), in the vicinity of Lages (Fig 1), were picked up between August 2007 and September 2012 by the Brazilian Environmental Agency (IBAMA) and sent to the Laboratory of Zoology and Parasitology of the Universidade do Planalto Catarinense (UNIPLAC).

The fecal samples were obtained directly from the wild cats' rectums at necropsy, frozen at 4°C, processed

within 24 h, and analyzed by two conventional methods: sedimentation (Hoffman et al. 1934) and flotation (Faust et al. 1938). Five grams of each sample were used in each method after homogenization (Ehlers et al. 2013). The larvae (L1) were observed under light

microscopy using 100X and 400X magnifications. The characteristics of L1, that is, the presence of a dorsal spine on the tail, allowed making a distinction from other nematode larvae (TRAVERSA AND DI CESARE 2013).

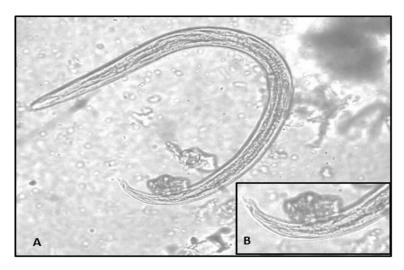
Fig. 1 Highways in Lages and neighboring towns in Santa Catarina, Brazil, where roadkills took place (2007-2012)



Results

Two out of 97 stray cats, one aged 6 months and one aged four years, were positive for *A. abstrusus* L1, yielding a 2.1% rate. Among wild cats, only *Puma yagouaroundi* was positive for this lungworm (14.3%). The larvae (Fig 2) were detected only by the sedimentation method, showing, on average, five L1 per slide.

Fig. 2 A - Aelurostrongylus abstrusus larva (100X). B – Dorsal spine on the tail (400X)



Discussion

This is the first systematic study specifically aimed at verifying the presence of A. abstrusus in the local stray cat population and among free-ranging wild cats, whose prevalence corresponds to 2.1% and 14.3%, respectively. A. abstrusus infection in domestic cats is a widely geographically distributed parasitic disease, considered to be both emergent and re-emergent. In Brazil, the prevalence rates among domestic cats range from 1.37% to 29.5% (Ohlweiler et al. 2010; Ehlers et al. 2013; Ramos et al. 2013), depending on the region.

Actually, a 2.1% prevalence rate among domestic cats is deemed to be low. The sample size, though, did not allow determining the prevalence among wild felids and, therefore, it was only possible to infer that there exist intermediate and/or paratenic hosts acting as vectors of transmission in both urban and wild areas of the investigated region. Both native and exotic mollusk

species have been infected with A. abstrusus larvae. Around 40% of 45 specimens of Achatina fulica Bowdich, 1822 studied in Brazil were infected by this parasite (Andrade-Porto et al. 2012). The gastropod A. fulica, native to southeastern Africa, was introduced into the American continent in 1939 and was brought to Brazil for commercial purposes in the 1980s. Nowadays, this snail is regarded as one of the most invasive pests, and is found in 23 out of 26 Brazilian states, acting an important vector of helminthic pathogens to animals and humans (Thiengo et al. 2008; Ohlweiler et al. 2010).

In Europe, most of the reports on the infection of domestic cats were published from 2000 onwards, showing different prevalence rates, depending on the cat population and on the geographic region. In Spain, the prevalence was 1% in domestic, stray, and farm cats (Miró et al. 2004); 28.7% in stray cats in Ibiza (Jefferies et al. 2010); 17.3% and 18.5% in central and southern Italy, respectively (Traversa et al. 2008b), and 2.9% in northern Italy (Spada et al. 2013); 5.6% in Romania (Mircean et al. 2010); and 14.5% in Hungary (Capári et al. 2013). In Denmark and in Germany, the prevalence rates were 5.6% (Taubert et al. 2009), but Barutzki and Schaper (2012) pointed out the disease was endemic in Germany.

A. abstrusus infection has been investigated all around the world: in Turkey (Tüzer et al. 2002), in Portugal (Waap et al. 2013), in Greece (Ellis et al. 2010), in Qatar (Abu-Madi et al. 2007), in Australia (Lacorcia et al. 2009), and in the United States (Lucio-Forster and Bowman 2011); with only one case being reported in Turkey (Tüzer et al. 2002), Greece (Ellis et al. 2010), and Albania (Knaus et al. 2011). Evidence of higher prevalence rates and new foci of A. abstrusus infection in European countries may be the result of the larger number of intermediate hosts, possibly due to global climate changes, in addition to the fact that most cats have access to the street, where they can come in contact with fecal matter from other cats and where they can ingest intermediate and/or paratenic hosts (Traversa et al. 2010).

In Thailand, A. abstrusus was reported in leopards (Panthera

pardus=55.55%), tigers (Panthera tigris=73.68%), and Asian leopard cats (Felis bengalensis =100%) (Patton and Rabinowitz 1994); in Tanzania, in lions (Panthera leo =7%) (Bjork et al. 2000). In Bolivia, the prevalence in wild felines (Oncifelis geoffroyi, Leopardus pardalis and Herpailurus vaguarondi) averaged 42.11%, corresponding to 50% (5/10) in L. pardalis and 37.5% (3/8) in O. geoffroyi (Fiorello et al. 2006). In Poland, the parasite was detected in Lyx *liyx* (21.1%) (Szczesna et al. 2006), and in eastern Siberia, González et al. (2007) detected larvae in feces of P. tigris altaica and F. bengalensis euptilurus.

A. abstrusus prevalence rates could be underestimated as infection may be asymptomatic and as costly and effective techniques are not always available in the routine practice of clinics and veterinary hospitals (Traversa and Guglielmini 2008; Traversa et al. 2008a; Lacorcia et al. 2009). L1 can remain viable in the feces at an ambient temperature of 21°C to 24°C for up to 45 days and at 4°C for 60 days (Gökpinar and Yildiz 2010), indicating that the appropriate disposal of feline waste plays a crucial role. Attention should be given to the life cycle and to the pathogenicity of this parasite, given that L1 are shed intermittently in the feces; hence, it is recommended that a 24-hour

sample be collected or that the collection occur three times daily (Barutzi and Schaper 2012), preventing falsenegative results, which are likely to be observed when the infection is still in the preparent period (Traversa et al. 2010).

The study of wild animals presents enormous difficulties, and reports on these species are scarcely available. This study could be undertaken because it was possible to pick up animals struck and killed by motor vehicles. These roadkills happen because urbanization has expanded hugely, thereby reducing wilderness areas, forcing felines to seek out food and sex partners elsewhere and to roam into urban spaces.

The distribution of helminthic pathogens, presence of clinical signs, physical epidemiological status, identification variables, and intermediate and/or paratenic hosts need to be investigated. Therefore, as a suggestion, public policies should be devised, including the detection of L1 by routine parasitological tests, especially in stray domestic cats, and getting these animals off the street. The flotation method was also used, as in other studies (Gaglio et al. 2008; Traversa et al. 2008b; Lucio-Forster and Bowman 2011; Barutzi and Schaper 2012); however, it is not efficient in detecting first-stage larvae in domestic and wild cats.

The identification of *A. abstrusus* raises a red flag about the presence of infected intermediate and/or paratenic hosts and about the importance of this parasitic disease even when respiratory clinical signs are absent. This is the first report of *A. abstrusus* in *Puma yagouaroundi* in Brazil.

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Compliance with ethical standards

All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations.

Conflict of interest

None

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