

## Review articles

# Current knowledge about *Aelurostrongylus abstrusus* biology and diagnostic

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**ABSTRACT.** Feline aelurostrongylosis, caused by the lungworm *Aelurostrongylus abstrusus*, is a parasitic disease with veterinary importance. The female hatches her eggs in the bronchioles and alveolar ducts, where the larva develop into adult worms. L1 larvae and adult nematodes cause pathological changes, typically inflammatory cell infiltrates in the bronchi and the lung parenchyma. The level of infection can range from asymptomatic to the presence of severe symptoms and may be fatal for cats. Although coprological and molecular diagnostic methods are useful for *A. abstrusus* detection, both techniques can give false negative results due to the presence of low concentrations of larvae in faeces and the use of inadequate diagnostic procedures. The present study describes the biology of *A. abstrusus*, particularly the factors influencing its infection and spread in intermediate and paratenic hosts, and the parasitic interactions between *A. abstrusus* and other pathogens.

**Key words:** *Aelurostrongylus abstrusus*, cat, lungworm, feline aelurostrongylosis

## Introduction

*Aelurostrongylus abstrusus* (Angiostrongylidae) is the most widespread feline lungworm, and one with a worldwide distribution [1]. Adult worms are localized in the alveolar ducts and the bronchioles. *A. abstrusus* infection can cause respiratory signs and often can be fatal for cats. *A. abstrusus* has an indirect life cycle which includes land molluscs, such as snails and slugs, as its intermediate hosts, and domestic and wild felids as its definitive hosts. Rodents, reptiles, amphibians and birds also act as paratenic hosts. Cats are infected by ingesting intermediate and paratenic hosts containing L3 larvae. The major problems regarding the treatment and prevention of feline aelurostrongylosis are the absence of clear diagnostic procedures and specific clinical signs. The Baermann technique is considered by some authors as a gold standard for lungworm larvae recovery from faeces; however, it can show false-negative results due to the low concentrations of the larvae present in faecal samples and the inadequacies of the diagnostic procedure. Moreover, *A. abstrusus* larvae closely resemble other feline lungworms, so misidentifications also occur

[1–3]. Another problem is a lack of data on host-parasite and parasite-parasite interactions between *A. abstrusus* and its definitive and intermediate hosts, and between *A. abstrusus* and other pathogens. The aim of this review is to summarise recent data regarding the biology and diagnosis of *A. abstrusus*.

## Distribution

Aelurostrongylosis has been identified in domestic cats throughout Europe (Germany, France, Italy, Spain, Portugal, Albania, Croatia, Turkey, Israel, Danish, England, and Belgium) [2–13] as well as in Africa [14], the USA, Australia and Brazil [15–17]. Its prevalence varies according to diagnostic method, region and lifestyle (i.e. feral/owned); the lowest prevalence has been registered in Croatia (0.3%), and the highest in Albania (50%) [1,7,8].

*A. abstrusus* has also been found in Europe [18] in two wild felids: the Eurasian lynx *Lynx lynx* L. [19] and the wild cat *Felis silvestris silvestris* Schreber, 1777 [20]; in Africa it has been found in the caracal *Caracal caracal* Schreber, 1776, lion

Table 1. List of gastropod molluscs, intermediate hosts for *A. abstrusus*

Superfamily	Family	Species	Infections
Achatinoidea	Achatinidae	<i>Achatina fulica</i>	natural
	Subulinidae	<i>Rumina decollata</i>	natural
Arionoidea	Arionidae	<i>Arion circumscriptus</i>	natural
		<i>A. lusitanicus</i>	natural
Enoidea	Enidae	<i>Chondrula septemdentata</i>	experimental
Helicoidea	Helicidae	<i>Helix aspersa</i>	natural, experimental
		<i>H. cavata</i>	experimental
		<i>Levantina hierosolyma</i>	experimental
		<i>Levantina caesareana</i>	experimental
		<i>Theba pisana</i>	experimental
	Hygromiidae	<i>Cernuella virgata</i>	natural
		<i>Helicella barbesiana</i>	experimental
		<i>H. vestalis joppensis</i>	experimental
		<i>Monacha syriaca</i>	experimental
Helicoidea	Helminthoglyptidae	<i>Helminthoglypta californicaeucsis</i>	experimental
		<i>H. tzickliuliniza</i>	experimental
		<i>H. arrosa</i>	experimental
Lymacoidea	Agriolimacidae	<i>Deroceras laeve</i> (syn. <i>Agriolimax laevis</i> )	experimental
	Limacidae	<i>Limax flavus</i>	experimental
Zonitoidea	Zonitidae	<i>Betinella nitellina</i>	experimental

Note: data summarized from references [10,25-31]

*Panthera leo* L., serval *Leptailurus serval* (Schreber, 1776) and cheetah *Acinonyx jubatus jubatus* Schreber, 1775 [21,22]. Some reports indicate that the *A. abstrusus* larvae identified in wild felid faeces may have been misidentified as other helminths, as mentioned by Elsheikha et al. [1]. Hence, more accurate data is needed on the spread of *A. abstrusus* in wild felids.

## History

In 1865, Leuckart described the roundworm *Ollulanus tricuspis* in the stomach of domestic cats, and stated that *O. tricuspis* larvae were often found in cysts in the intestines, as well as in the lungs. In 1890, Mueller described lungworm from the cat, which he named *Strongylus pusillus*. This name was afterwards found to be a homonym of Rudolphi's *Strongylus pusillus*, and Railliet proposed the new specific name of *S. abstrusus* instead. In 1905, Kamensky removed the species *S. abstrusus* from the genus *Strongylus*, and placed it in a new genus, *Protostrongylus*, on the basis of its morphology. In 1907, Railliet and Henry apparently ignorant of Kamensky's work, placed the worm in the genus *Synthetocaulus*. In 1926, Leiper suppressed the

genus *Synthetocaulus*, as it was a synonym of *Protostrongylus*, Kamensky 1905. The following year, Cameron transferred the parasite to a new genus, *Aelurostrongylus*, on the basis of further morphological study [23,24].

## Life cycle

The nematode *A. abstrusus* has an indirect life cycle which includes the domestic cat and wild felids as definitive hosts, and terrestrial molluscs (snails and slugs) as intermediate hosts; small vertebrates such as rodents, lizards and frogs are paratenic hosts [1,2]. Adult worms are localized in the alveolar ducts and terminal bronchioles of the definitive host. The female hatches the eggs in lung parenchyma and small blood vessels, where the first stage larvae develop. The L1 larvae migrate via the bronchi and trachea to the pharynx, where they are then swallowed and passed in the faeces to the environment. L1 larvae develop into L3 larvae in the intermediate hosts, i.e. the land molluscs [1].

## Development in intermediate hosts

The lungworm *A. abstrusus* can infect a wide

spectrum of molluscs [10,25–31] (Table 1). There is some confusion regarding how L1 larvae can infect snails. Hobmaier and Hobmaier [32] first supposed that L1 penetrate the snail foot. Some studies of the life cycle of the rat lungworm *Angiostrongylus cantonensis* show that snails become infected by the ingestion of L1; however, further experimental studies of the *A. abstrusus* life cycle, particularly its development in snails, show that after two to four hours of contact with snails, the L1 larvae invade the sole of the snail foot and migrate into the deep layers of the foot and visceral organs over the next 24–48 hours.

The development of L1 larvae to the L3 stage depends on temperature: experimental studies show that in the temperature range 18.8–29.5°C, about 50% of *A. abstrusus* larvae reach the infective stage, while only 17.8% of larvae completed their development at temperatures of 6.7–22°C. The first moult (L2) occurs about 6–15 days after infection and the second moult (L3) about 3–6 days later in the temperature range 18.8–29.5°C. However, L3 larvae emerge about 37 days after infection at a temperature of 6.7–22°C.

Although *A. abstrusus* larvae are found in both the foot and viscera of snails, high concentrations of L3 larvae can be found in the foot [33,34]. Climatic conditions such as temperature, moisture and water availability highly influence the abundance and activity of the intermediate hosts [34]. Paratenic hosts play an important role in the life cycle of *A. abstrusus*. Hobmaier [35] found that molluscs are eaten by animals such as frogs, toads, lizards and snakes, and that some species of birds and rodents may accumulate L3 larvae, thus becoming an important link with the disease in the cat. Hamilton and McCaw [36] found that no further development of L1 larvae occurs after experimental ingestion in mice; they concluded that the mouse is not an intermediate host for *A. abstrusus*. Hobmaier [35] suggests that mice are paratenic hosts only, and attribute the infective stages found in mice to the result of feeding mice and white rats with infected molluscs [35]. Cesare et al. [34] note that the infection of paratenic and definitive hosts by *A. abstrusus* increases during warm periods of the year, due to the high activity of slugs at warm temperatures.

### Development in definitive hosts

When infected molluscs or paratenic hosts are

eaten by cats, the L3 larvae migrate via the mucosae of the digestive system and via the lymphatic system to the lungs, where they undergo the third and fourth moults and mature to adult worms [1,2]. The prepatent period in the cat was about 35–48 days. Infected cats produce large numbers of L1 larvae in their faeces. Peak larval output was observed during the period 60–120 days post infection [37–39].

### Symptoms and pathogenicity

The clinical symptoms and damage caused by *A. abstrusus* depend on several factors such as worm burden, age of the feline host, presence of other diseases and infection [40]. Clinical signs can be asymptomatic, mild (sporadic cough, sneezing), moderate (cough, dyspnoea, anorexia) or severe (cachexia, abdominal breathing, muco-purulent discharge) [4,11,40–42]. However, the pathology and clinical signs of feline aelurostrongylosis are unspecific and not limited to respiratory signs and anorexia [43–46]. Ascites and enteritis associated with heavy *A. abstrusus* infection have been observed in a two-month-old kitten [47]. *Aelurostrongylus* infection can also be accompanied with other parasites or bacterial infection, with one case of concurrent infection of *A. abstrusus* and *Salmonella typhimurium* being registered in a 14-week-old kitten [16].

Mixed infections of *A. abstrusus* with other lungworms have been registered in cats. Two cases of mixed *A. abstrusus* and *Troglostrongylus brevior* infection were registered in kittens from Italy: one kitten demonstrated severe respiratory signs while the other presented a subclinical form of infection [48]. In addition, two cases were also registered in Greece [49]. A case of mixed infection by *A. abstrusus* with *Oslerus rostratus* was reported in a one-year-old cat in Spain with severe respiratory signs [50]. A case of a mixed infection by lungworms *A. abstrusus* and *T. brevior* and the cardio-pulmonary roundworm *Angiostrongylus chabaudi* was reported in a cat from Italy [51].

Cats infected with *A. abstrusus* show a wide range of clinical signs from asymptomatic to severe symptoms, while *T. brevior* infection usually causes severe symptoms which is often fatal for cats [48–51]. There is a lack data about the pathogenicity and distribution of *A. chabaudi* in domestic cats. The mode of interaction between helminths and the host-parasite interactions in cats co-infected by

different lungworms is unknown. In co-infections with two or more parasites, the burden of one or both infectious agents may be decreased or increased, or one may be suppressed and the other increased. Moreover mixed infections can influence the pathology of infections [52]. So in the case of co-infections, it is necessary to investigate not only the pathogenicity, biology and host-specific interactions of lungworm parasites, but also the interactions between lungworms of the same species, as well as between lungworms and other parasitic and bacterial agents.

### Histopathological changes

Histopathological changes occur due to the hatching of eggs and migration of L1 larvae, which stimulates inflammation in arteries, bronchioles and alveoli. The histological changes commonly observed in lung parenchyma include the presence of inflammatory cell infiltrates with neutrophils and macrophages, and rarely with eosinophils. Lesions in the bronchi include inflammatory cell infiltrates in bronchiolar walls and the lumina, destruction of the bronchiolar epithelium and hyperplasia of bronchus-associated lymphoid tissue and peribronchial glands. Hyperplasia of lymphoid tissue also appears in tracheobronchial lymph nodes. Pulmonary artery histological changes include the presence of inflammatory cell infiltrates localised in the subintimal area [43–45,53,54].

### Diagnostic procedures

Diagnostic procedures of *A. abstrusus* infections include standard coprological examination techniques, histological examination, and PCR and IFA tests [1,55].

Direct coprological examination methods are simplest techniques for L1 larva detection in faeces. However, the sensitivity and efficiency of such techniques is highly dependent on the type of solution, its specific gravity and duration of sample processing. Flotation solutions with a specific gravity of 1.20 floated significantly fewer lungworm larvae than those with a higher specific gravity [55]. Therefore, frequently-used standard flotation and sedimentation techniques are not effective for the diagnosis of *A. abstrusus*.

Only four coproscopical examination methods are suitable for *A. abstrusus* diagnosis: the Baermann technique, McMaster method,

Wisconsin technique and FLOTAC method [55,56]. The Baermann technique is regarded as the most sensitive technique for the detection of live roundworm larvae of various species in faeces. It also has been conducted as a „gold standard” for *A. abstrusus* diagnosis [56,57]. Later, the Baermann technique was considered a highly-sensitive method for the detection of *A. abstrusus* larvae in lungs compared to necropsy and histological examination of lung tissue. This method is also used for cytological examination of bronchoalveolar lavage fluid. Both cytological examinations techniques have equal sensitivity compared to standard necropsy and histological examination; however, by using a combination of both cytological techniques, sensitivity can be increased by 9.1–18.1% [57,58].

The McMaster flotation technique has less sensitivity for *A. abstrusus* L1 detection in faeces compared to the Baermann and Wisconsin techniques [56]. However, this method has high sensitivity for most helminth species (roundworms, flatworms), and the efficacy of this technique strongly depends on flotation time, specific gravity of the solution and sample dilution [59].

The Wisconsin flotation centrifugation technique is more rarely used for helminth larvae recovery from faeces than the other methods mentioned above. However, this method has higher sensitivity than the McMaster technique for the recovery of *A. abstrusus* L1 larvae [55].

The FLOTAC technique is a new multivalent technique which has high sensitivity for most helminth and protozoan species [59]. The method was designed by Gringoly in 2006 for human and animal medicine [60]. This is an egg-count technique conducted with Flotac apparatus: a cylindrical shaped device holding 12 sample flotation chambers, each measuring 5 ml with two ruled grids. Each grid contains 12 equidistant ruled lines. The method uses a battery of flotation solutions with specific gravities varying from 1.2 to 1.45 gr/l. The FLOTAC technique has also been used for *A. abstrusus* diagnosis, and has been found to demonstrate greater sensitivity than the Baermann, MacMaster and Wisconsin techniques [59]. However, all coproscopical methods can show false negative results, due to the fact that L1 shedding is discontinuous [61]. Therefore, alternative methods such as bronchoalveolar lavage, molecular diagnostic and IFA tests are better tools for the diagnosis of *A. abstrusus* infection.

Another procedure which can be used for the

diagnosis of *A. abstrusus* infection is bronchoalveolar lavage, a procedure where cells are retrieved from the lungs for evaluation. The removed bronchoalveolar fluid (BALF) is investigated under a stereomicroscope for the presence of L1 larvae. The efficacy of the method can be improved by using Baermann solution for the BALF examination [61].

Recently, PCR techniques have been developed for *A. abstrusus* diagnosis. Firstly, a nested PCR is performed to identify rDNA. This method has been validated both for pharyngeal swab samples and for faecal samples comprising faeces, Baermann sediment and floatation supernatant. This method has 100% specificity and 97% sensitivity, it also permits *A. abstrusus* identification in asymptomatic cats and cats showing negative result in classical diagnostic methods [62]. To identify two species of feline lungworm, *A. abstrusus* and *T. brevior*, a duplex PCR diagnostic method has been developed using species-specific forward primer sets for the ITS-2 region [63]. Multiplex PCR has also been used to successfully identify three species: *A. abstrusus* and *T. brevior* and the heartworm *A. chabaudi* [64]. Molecular diagnostic methods have higher specificity and sensitivity than coprological and histological methods, and thus allow species which have similar morphological characters to be identified more precisely [62]. Serological analyses can also be used for *A. abstrusus* detection. For example, indirect immunofluorescence antibody assay has been used for the detection of *A. abstrusus* antibodies in the sera of infected cats; this method has high sensitivity and does not cross react with serum from other parasitic nematodes [65].

Another newly-developed method for detecting *A. abstrusus* antibodies in sera samples is based on enzyme-linked immunosorbent assay (ELISA). The approach has a sensitivity of 88.2–100% and specificity of 68.1–90%. However, the method has been limited by cross-reactivity with *Toxocara cati*, *Capillaria*, *Taenia* and hookworms [66]. Despite this, the development and ongoing improvement of ELISA assays for the diagnosis of *A. abstrusus* represents a major tool for the diagnosis and investigation of aelurostrongylosis in cats.

### Additional diagnostic methods

Other laboratory techniques such as blood analyses, sonography and radiography are also useful for *A. abstrusus* diagnosis [1,67,68].

Computed tomography (CT) is a frequently-used method for diagnosing pulmonary diseases in animals. CT allows the visualisation of thoracic structures and quantification of lesion severity. Radiographic image depends on the period of infection and worm abundance. In the early stage of infection, bronchial thickening and small, poorly-defined nodules can be seen. Further multiple pulmonary nodules appear throughout the lungs. Subpleural thickening, interface signs and ground-glass opacity formation can be found in local regions or spread throughout the lungs according to severity of infection. At later stages of infection, CT shows a generalized, structured, nodular bronchointerstitial and interstitial pulmonary pattern of varying severity and a high suspicion of tracheobronchial lymphadenomegaly. No peripherally accentuated pattern is observed in affected animals due to the localisation of *A. abstrusus* worms and egg production [40,68].

Clinical blood analysis of cats affected with *A. abstrusus* shows eosinophilia, lymphocytosis and leucocytosis, rare basophilia and monocytosis [41,69].

Blood gas analysis shows respiratory acidosis and hypoventilation due to airway obstruction of bronchioles and alveolar canals by adult worms and L1 larvae [70].

The diagnosis of *A. abstrusus* is complicated by the absence of specific clinical signs and the limitations of diagnostic methods [1]. All coproscopic examination methods used for *A. abstrusus* L1 larva detection in faeces can show false-negative results due to low larva concentration in the faecal sample and inadequate morphological differentiation from other helminth larvae [55,62]. The efficiency of coprological examination is highly dependent on sample freshness, accuracy of the procedure and the period of sample processing. PCR technique is also can show a false negative result due to inadequate DNA extraction [62]. However, the two techniques are the most effective tools for the diagnosis of *A. abstrusus*.

### Conclusions

Literature data shows that *A. abstrusus* affects a wide spectrum of slugs and snails as intermediate hosts, and domestic and wild felids as definitive hosts in different continents [1,24,25]. Paratenic hosts such as birds, rodents, amphibians and reptiles participate in its spread. However, little is known of

the factors influencing its transmission between intermediate and paratenic hosts, and data on the immune response caused by *A. abstrusus* in definitive hosts is incomplete. Furthermore, the interactions between *A. abstrusus* and other pathogens such as bacteria and parasites are unclear [1]. New serological methods of diagnosis require further development. Therefore, more knowledge is required of the parasite-host and parasite-parasite interactions involving *A. abstrusus* before the diagnostic procedures and treatment of aelurostrongylosis can be further developed and modernised.

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