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Transmammary transmission of *Troglostrongylus brevior* feline lungworm: a lesson from our gardens

Marcos Antônio Bezerra-Santos, Jairo Alfonso Mendoza-Roldan, Francesca Abramo, Riccardo Paolo Lia, Viviana Domenica Tarallo, Harold Salant, Emanuele Brianti, Gad Baneth, Domenico Otranto

**Abstract**

Feline lungworms such as *Aerulostrongylus abstrusus* and *Troglostrongylus brevior* are snail-borne pathogens causing respiratory disease in domestic cats. Paratenic hosts such as rodents and reptiles have also been implicated in the epidemiology of these parasites. Although *A. abstrusus* has been recognized for a long time as the most prevalent lungworm among cats worldwide, *T. brevior* is of major concern in kittens. Bearing in mind that disease due to *T. brevior* occurs mainly in pediatric patients younger than 6 months of age, the diagnosis of this parasite in two kittens presenting severe respiratory disease from the garden of one of the authors inspired us to investigate the potential routes of transmission for *T. brevior* in domestic cats. Of the three queens (A, B and C) that delivered kittens (*n* = 8), only cat A was positive for *T. brevior*, presenting her two kittens severe respiratory clinical signs, which lead to the exitus in one of them, 18 days of age. In addition, three kittens, the offspring of queen B, turned to be positive at the coprological examination after suckling from queen A, whereas those from queen C (that suckled only on their own mother) remained negative. A series of coprological, histological and molecular tests were conducted to confirm the presence of *T. brevior* in the patients as well as in the other cats cohabiting the same garden. Adult nematodes were retrieved from the trachea and bronchi of the dead kitten (kitten 1A), and larvae at the histology of the lung and liver parenchyma associated with bronco pneumonitis and lymphocytic pericholangitis, respectively. *Cornu aspersum* (*n* = 60), *Eobania vermiculata* (*n* = 30) snails (intermediate hosts) as well as lizards and rats (potential paratenic hosts) were collected from the same garden and processed through tissue digestion and molecular detection. *Troglostrongylus brevior* larvae were recovered through tissue digestion from two *C. aspersum* (33.33 %) and it was confirmed by PCR-sequencing approach, which also detected *T. brevior* DNA in the liver and lungs of one rat and in the coelomatic cavity of one gecko lizard. During the COVID-19 lockdown, when scientists spent more time at home, we grasp the opportunity to decipher *T. brevior* biology and ecology starting in a small ecological niche, such as the garden of our house. Data herein presented led us to suggest: i) the transmammary transmission of *T. brevior* in domestic cats; ii) the role of intermediate and paratenic hosts (including reptiles) in the epidemiology of the infection which they transmit; as well as iii) the importance of observational parasitology in studying any event that certainly occurs in small ecological niches, as it could be in our home gardens.

1. Introduction

Amongst snail-borne parasites, feline lungworms (superfamily Metastongyloidea) cause major respiratory diseases in domestic and wild felids, with *Aerulostrongylus abstrusus* being the most prevalent worldwide (Elsheikha et al., 2016). Another species of feline lungworm, *Troglostrongylus brevior* (Gerichter, 1949) has for long time been regarded as of minor importance, most likely because it was overlooked by
parasitologists (Brianti et al., 2012) and eventually considered typical of wild cats (Traversa and Di Cesare, 2013; Diakou et al., 2015). However, in the last decade, *T. brevior* has gained substantial attention of the scientific community after being identified as causative agent of severe broncho-pulmonary disease in a large number of cat population, mostly in kittens and young cats (Brianti et al., 2012, 2013; Di Cesare et al., 2014; Cavaleri et al., 2018; Salant et al., 2020). For instance, a study performed in some European countries recorded that 19.5% of the cats that scored positive for lungworms were infected by *T. brevior* (Giannelli et al., 2017).

Though a comprehensive picture of the distribution of this parasite is still far from being achieved, it has been reported in domestic cats from many countries in many countries, including Spain (Jefferies et al., 2010; Giannelli et al., 2017), Italy (Brianti et al., 2012, 2013; Tamponi et al., 2014, 2017; Giannelli et al., 2017; Cavaleri et al., 2018; Traversa et al., 2019), Greece (Diakou et al., 2014, 2015), Cyprus (Diakou et al., 2017), Bulgaria (Giannelli et al., 2017) and Romania (Deak et al., 2017). At the same time, *T. brevior* has been recorded in wild felids (*Felis silvestris silvestris*) from Italy (Palsено et al., 2014; Napoli et al., 2016) and Greece (Litiς et al., 2017), and in Lynx lynx from Bosnia and Herzegovina (Aliche et al., 2015) suggesting the circulation of this parasite in endemic areas. The clinical relevance of this lungworm species is mainly observed in cats younger than 12 months, in which it frequently causes severe illness of the lower respiratory tract, with the animals presenting cough, dyspnea and tachypnea, as well as nonspecific clinical signs (e.g. partial or complete anorexia, hyperthermia or hypothermia, dehydration, ocular and/or nasal discharge, sneezing, pulmonary hypertension, poor body conditions, and lethargy) (Crisi et al., 2015, 2018). Conversely, adult individuals commonly present subclinical infection (Crisi et al., 2018).

*Aerulostrongylus abstrusus* and *T. brevior* present similar biological cycles, with molluscs (i.e. snails and slugs) of various species (e.g. *Chondrula septemdentata*, *Helicella barbesiana*, *Helicella ustalis*, *Limax aspersum*, *Monaca syriaca*, *Retinella nitellina*, *Theba pisana*, and *Cornu aspersum*) regarded as intermediate hosts, and rodents, birds and amphibians acting as paratenic hosts (Gerichter, 1949; Giannelli et al., 2014). While the role of reptiles as paratenic host has been demonstrated for *A. abstrusus* (Hobmaier, 1937; Anderson, 2000), no information is available for *T. brevior*. This could be due to the scant number of parasitological studies available for reptiles, in spite of their role as source of many zoonotic agents (Mendoza-Roldan et al., 2020). Paratenic hosts have a relevant role in the epidemiology of lungworm disease, as these small vertebrates are commonly preyed upon by domestic cats, and act as a source of infection (Crisi et al., 2018; Colella et al., 2019). Alternative transmission pathways for *A. abstrusus* and *T. brevior* have been suggested. For example, the shedding of infective third stage larvae in the environment via the mucus, or contamination of water by larvae released by dead snails have been demonstrated (Giannelli et al., 2015), and snail to snail transmission of infective larval stage has been described as intermediates (Colella et al., 2015).

While *A. abstrusus* affects primarily adult cats, *T. brevior* is of pediatriac concern, as most studies reporting these lungworm species found kittens to be mostly affected by severe respiratory disease (Salanti et al., 2020), which in many cases is fatal (Brianti et al., 2013; Di Cesare et al., 2014). This is further confirmed by an epidemiological study performed in cats from different age groups in Italy, which demonstrated that in young cats and kittens (i.e., ≤ 6 months) *T. brevior* is the most frequent lungworm detected (i.e. 67.3%; 44/65), whereas in individuals aging 6–24 months, a low prevalence was observed (Cavaleri et al., 2018).

**2. Material and methods**

### 2.1. Sampling procedures

Two 18 days old kittens (1 female, 1 male, herein identified as 1A and 2A) presenting severe respiratory disease were observed in one of the authors’ garden. A rectal swab was used to sample both kittens (1A and 2A) and metastrongyloid larvae were detected. On the following day, the female kitten (1A) was found dead, while the male (2A) was taken away by its mother to an unknown location, therefore the outcome for this individual was not known to the authors. A new rectal swab and a pharyngeal swab were taken from the dead kitten for viral (Feline Herpes Virus 1 – FHV1, Feline Calicivirus – FCV) and bacterial tests, and a full necropsy was then performed on the animal.

Liver, spleen, lungs, heart, stomach, intestine and mesenteric lymph node samples were fixed in 10 % buffered formalin, embedded in paraffin, and cut at 5 μm sections (Canene-Adams, 2013). Thereafter, sections were deparaffinized, stained with hematoxilin and eosin and observed under a light microscope. Additionally, fragments of the same organs (except lungs) were submitted to artificial peptic digestion (Giannelli et al., 2015) and PCR screening (see molecular analysis heading).

Aiming to perform an epidemiological investigation on these clinical cases (two kittens), all cats inhabiting the house, including the mother (queen A) of the pediatric patients, two other queens (B and C), one adult male, and the offspring of the female queen B (3 kittens identified as 1B, 2B and 3B) and C (3 kittens identified as 1C, 2C and 3C) were tested for the presence of lungworms in their feces by the Baermann technique (MAFF, 1986).

### 2.2. Investigation on intermediate and paratenic hosts

Intermediate (*Cornu aspersum* and *E. vermiculata*) and paratenic hosts (rats and lizards) were collected in the garden where the study was performed. *Cornu aspersum* (*n* = 60) and *E. vermiculata* (*n* = 30) snails were artificially digested for the retrieval of larvae as previously described (Colella et al., 2015). Two rats (*Rattus norvegicus*) found dead were collected in the garden. In addition, lizards (*Podarcis siculus*, *n* = 1; *Tarentola mauritanica*, *n* = 2) were captured and humanely euthanized by cervical dislocation. These paratenic hosts were dissected and their organs (i.e. brain, diaphragm, heart, kidneys, liver, lungs, spleen and skeletal muscle) were individually digested. PCR for the detection of nematode DNA was performed in snails from positive pools and in the organs of paratenic hosts (See molecular analysis heading).

### 2.3. Molecular analysis

Genomic DNA extraction of the dead kitten organs, snails, and paratenic hosts organs was performed using a commercial kit (QiAamp DNA Micro Kit; Qiagen, Hilden, Germany) following the manufacturer’s instructions. Therefore, samples were screened by employing a duplex PCR using the forward primers *TrogloF* (5’-GCCATTGGAATCTTGCA-3’), *AeluroF* (5’-GCACTTGCAAATCTTGCA3’), and the single reverse primer *MedR* (5’-TAAAGCATATATATATTGGC3’), which amplify the ITS-2 region of different sizes (≤ 220 bp for *A. abstrusus*; 370 bp for *T. brevior*) (Annoscia et al., 2014). The same primers were used to purify amplifications, which were sequenced in both directions, using the Taq Dye
Deoxy Terminator Cycle Sequencing Kit v.2 (Applied Biosystems, Foster City, California, USA) in an automated sequencer (ABI-PRISM 377; Applied Biosystems, Foster City, California, USA). The obtained sequences were edited and aligned using the Mega7 software and compared with those available in the GenBank database, using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/BLAST.cgi).

3. Results

Rectal and pharyngeal swabs of dead kitten scored negative for both viruses (FHV1, FCV) at real time PCR. Microbiological tests were negative for Chlamydia spp. and Bordetella spp., but positive for Pasteurella spp. At necropsy, adult lungworms (n = 35) morphologically identified as *T. brevior* (Gerichter, 1949; Brianti et al., 2012) were recovered from the trachea and bronchi. Briefly, parasites were cylindrical, with an inflated cuticle thrown in folds, a club-shaped and short esophagus, a weakly developed small stoma, and a large excretory gland following the first third of the esophagus (Fig. 1). Female worms (n = 19) presented an average length of 9.55 mm and an average width of 0.43 mm, while male parasites (n = 16) were ~5.97 mm in length and ~0.32 mm in width. Additionally, larvae of the same lungworm species were isolated from the content of the stomach, esophagus and intestine, as well as in the feces through Baermann’s technique. Diffuse bronchopneumonia was the only gross abnormality observed, all the other organs were unremarkable.

Lung histopathology revealed the presence of numerous adult lungworms in the bronchi (Fig. 2A, B) and a few larvae in the parenchyma (Fig. 2C); entire lobes were affected by severe catarrhal-purulent bronchopneumonia (Fig. 2D). Lymphocytic pericholangitis was seen in the liver and a coiled larva was found within a neutrophilic focal infiltrate in the liver parenchyma (Fig. 3). No histopathological alteration neither parasites/larvae were observed from the other organs examined.

First-stage larvae (L1) of *T. brevior* were retrieved from the feces of queen cat A, and from 1 month-old kittens 1B, 2B and 3B (i.e. the offspring of queen B), which fed on milk from queen A, being all asymptomatic. Queens B and C, as well as the male cat and the kittens of queen C (1C, 2C, 3C) scored negative for nematode larvae in the feces (Table 1). Retrieved L1 (average length \(= 326.8 \mu m \pm 14.8 \mu m\); average width \(= 16.6 \mu m \pm 0.5 \mu m\)) presented rhabditoid esophagus, various granulated cells composing the intestine, and a pointed tail with cuticular dorsal and ventral spines (Fig. 4).

Larvae were not detected at artificial digestion of the organs; however, molecular analysis confirmed the histological findings of *T. brevior* in the liver and lungs. At artificial digestion, nematode larvae were recovered from two out of 60 (3.33 %) *C. aspersum*, which was
confirmed as *T. brevior* by PCR-sequencing approach. In paratenic hosts, larvae of this lungworm were not detected through artificial digestion, however, PCR analysis detected *T. brevior* DNA in the liver and lungs in one rat (*R. norvegicus*), and in the coelomatic cavity of one gekko lizard (*T. mauritanica*). PCR yielded amplicons of the expected size (i.e. 370bp) and sequence obtained showed a high homology (100 %) at BLAST analysis, with *T. brevior* DNA sequences available in Genbank (accession number: KF241978.1, KT818789.1, MH537789.1, MH780055.1). Obtained *T. brevior* sequences were submitted to GenBank under the accession number: MT772032 to MT772037.

4. Discussion

This study assessed the transmammary transmission of *T. brevior* in kittens, as well as the epidemiologic chain of the infection in paratenic (rats, lizards) and intermediate hosts (*C. aspersum* snails) that may occur in a garden. In addition, this is the first report of lizards as potential paratenic hosts for this parasite. Severe respiratory disease associated to *T. brevior* adults in two 18-day-old kittens from the infected cat (queen A), as well as the positive coprological tests in the kittens born from queen B, negative for *T. brevior*, following suckling milk from queen A, strongly support that the infection occurred by feeding of milk from the positive cat. This transmission pathway was previously suggested in a study, and sequence obtained showed a high homology (100 %) at BLAST analysis, with *T. brevior* DNA sequences available in Genbank (accession number: KF241978.1, KT818789.1, MH537789.1, MH780055.1). Obtained *T. brevior* sequences were submitted to GenBank under the accession number: MT772032 to MT772037.

The presence of *T. brevior* DNA in the liver and lungs of a rat, as well as in the coelomatic cavity of a gecko lizard, confirms the participation of these small vertebrates in the transmission cycle of this lungworm. It is worth noticing that the positive lizard was captured in a box full of snails, which suggests that this paratenic host could have become infected through the ingestion of snails or, merely, by contact with L3 shed in the mucus of those snails (Giannelli et al., 2015). Likewise, the same treatment has also been effective against *T. brevior* and *A. abstrusus* mixed infection in a ~2-month-old kitten (Di Cesare et al., 2015). However, the safety of most chemicals against feline lungworms has not been guaranteed for kittens or pregnant females (Cavalera et al., 2018).

The presence of *T. brevior* DNA in the liver and lungs of a rat, as well as in the coelomatic cavity of a gecko lizard, confirms the participation of these small vertebrates in the transmission cycle of this lungworm. It is worth noticing that the positive lizard was captured in a box full of snails, which suggests that this paratenic host could have become infected through the ingestion of snails or, merely, by contact with L3 shed in the mucus of those snails (Giannelli et al., 2015). Despite being known as paratenic hosts for lungworms, no records of rats infected with *T. brevior* has been reported. However, an encysted larva of this nematode was retrieved from the lung surface of one mouse experimentally infected (Gerichter, 1949). Additionally, mice have been previously proven to be paratenic hosts with an important role in the transmission of *A. abstrusus*, as cats acquired this nematode after feeding on...
experimentally infected mice (Colella et al., 2019). In the same study, third stage larvae of *A. abstrusus* were recovered from the liver, spleen, brain, skeletal muscle and gastrointestinal tissues of mice, demonstrating that this nematode migrates to various anatomical organs in the host (Colella et al., 2019). Likewise, our findings suggest the same pattern of migration for *T. brevior* in rats and lizards, demonstrating that these hosts have a relevant role in the epidemiology of feline lungworm disease, as they may be the main source of *T. brevior* infection to adult cats. In particular, cats present an increased hunting activity of mice and lizards during pregnancy or lactation due to the increased need for protein intake, therefore resulting in a greater risk of infection to lungworms through predation (Brianti et al., 2013).

The presence of infected intermediate hosts (e.g. *C. aspersum* snails) in the garden, highlights the importance of these mollusks in the epidemiology of the disease, although cats usually do not feed on snails, but rather on paratenic hosts, such as rats and lizards (Gerichter, 1949; Woods et al., 2003; Giannelli et al., 2014). Despite not being considered the main direct source of infection to domestic cats, intermediate hosts have an essential role in the epidemiological chain of *T. brevior*, as they are predated upon by paratenic hosts, such as rats (Hadfield and Saufier, 2009). Under the above circumstances, infective third stage larvae released from snails in the environment, or in water soon after they drown, may enhance risk of *T. brevior* transmission to cats and paratenic hosts (Giannelli et al., 2015). This is further suggested in our study, as the presence of *T. brevior* was confirmed in a rat and a lizard collected at the same site where the clinical cases occurred and a high population of snails was present.

In parasitology, constant interactions among hosts, parasites and their vectors occur in small ecological niches such as a home garden. Observational approaches should be prompted to further investigate scientific events that would be further confirmed under experimental conditions. As new scientific discoveries require time and reproducible, confirmatory, results, very often we rely much more on laboratory-based observations than on events that occur in nature. Without any doubts, during the COVID-19 lockdown, scientists spent more time at home, which might have allowed to decipher observational events, such as the one herein described. This provides a good lesson for planning future research in many fields of science, including parasitology.

Fig. 3. A) Coiled larva (*) within a focal accumulation of neutrophils in a liver lobule around a centrilobular vein (200X magnification). B) Portal tract, mild lymphocytic pericholangitis (400X magnification).
epidemiological chain of gardens. Moreover, this study brings important knowledge on the any event that occurs in small ecological niches, such as our home.

** Positive at rectal swab.

Fig. 4. First-stage larva recovered from the feces of the cats herein evaluated.

### 5. Conclusions

The findings herein reported support: i) the hypothesis that *T. brevior* could be vertically transmitted through the transmammary route; ii) the role of intermediate and paratenic hosts in the epidemiology of the disease in cats (including the first evidence of reptiles as paratenic hosts); and iii) the importance of observational parasitology in studying any event that occurs in small ecological niches, such as our home gardens. Moreover, this study brings important knowledge on the epidemiological chain of *T. brevior* transmission in domestic cats, drawing attention for the need of preventive measures such as early diagnosis and suitable age-related treatment of pregnant queens and kittens.

### Ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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