International Journal for Parasitology 49 (2019) 449–453

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Shedding of feline lungworm larvae and their infectivity to snail intermediate hosts after anthelmintic treatment

Maria Alfonsa Cavalera [a,1], Vito Colella [a,1], Ettore Napoli [b], Francesca Arfuso [b], Rossella Panarese [a], Emanuele Brianti [b], Domenico Otranto [a,*]

[a] Dipartimento di Medicina Veterinaria, Università di Bari, 70010, Valenzano, Bari, Italy
[b] Dipartimento di Scienze Veterinarie, Università di Messina, 98168, Messina, Italy

ARTICLE INFO

Article history:
Received 12 November 2018
Received in revised form 19 December 2018
Accepted 20 December 2018
Available online 21 March 2019

Keywords:
Lungworms
Snails
Infectivity
Larvae
Aelurostrongylus abstrusus
Troglostrongylus brevior
Anthelmintic treatment

ABSTRACT

Aelurostrongylus abstrusus and *Troglostrongylus brevior* are snail-transmitted helminths causing respiratory diseases in infected cats. The shedding of feline lungworm L1s and their infectivity to the snail intermediate host, after administration of anthelminthic products to cats, are poorly documented. To assess the efficacy of 8.3% fipronil, 10% (*S*)-methoprene, 0.4% eprinomectin and 8.3% praziquantel (i.e. eprinomectin formulation) and 10% imidacloprid/1% moxidectin (i.e. moxidectin formulation) against these nematodes and to determine the number of days post-treatment until viable L1s are released in the faeces, 384 animals were screened by faecal examination. Of the 54 positive animals (i.e., 14.1%; 7.3% *A. abstrusus*, 6.2% *T. brevior* and 0.5% coinfected), 36 were randomly allocated to four groups. Groups A and B were composed of cats positive for *T. brevior* and treated with the eprinomectin and with the moxidectin formulations, respectively, whereas cats in groups C and D were positive to *A. abstrusus* and treated with the eprinomectin and the moxidectin formulations, respectively. Prior to and every day after treatment, faecal samples were analysed by the Baermann technique and the number of larvae per gram of faeces determined, and again four weeks after treatment, to assess the efficacy of a single administration of the products. In addition, to evaluate the pre- and post-treatment infectivity of L1s to snail intermediate hosts, one/two snails per cat were infected with 100 L1s collected from the faeces of enrolled animals and then digested 28 days p.i. Based on L1s faecal counts, the efficacy of the eprinomectin and the moxidectin formulations at 28 days was 100% for both *A. abstrusus* and *T. brevior,* with a mean number of days of 7.9 ± 1.2 in group A, 7.8 ± 1.9 in B, 6.9 ± 1.6 in C and 8.9 ± 2.0 in D to become negative. Following the artificial digestion, active L3s of *T. brevior* and *A. abstrusus* were found in 160 (87.4%) experimentally infected snails. The results of this study demonstrate that a single administration of the two formulations is effective in the treatment of *A. abstrusus* and *T. brevior* infections and that during the post-treatment period live L1s are shed for up to 8.9 ± 2.0 days. L1s of both lungworm species released in the faeces after drug administration are still able to reach the infective larval stage in the infected snails. Hence, preventative measures after the treatment of infected animals should include keeping cats indoors and disposal of their faeces for approximately 10 days to avoid environmental contamination and infection of gastropod intermediate hosts.

1. Introduction

Feline lungworms are gastropod-transmitted helminths parasitizing the respiratory tract of cats across Europe (Giannelli et al., 2017). In infected animals, *Aelurostrongylus abstrusus* (Strongylida, Angiostrongylidae) colonizes the bronchioles and alveolar ducts, whereas *Troglostrongylus brevior* (Strongylida, Crenosomatidae) is found in the bronchii and bronchioles (Brianti et al., 2014). The outcomes of lungworm infections on the health of domestic animals range from no clinical signs to cough and dyspnoea leading to fatalities in young animals (Brianti et al., 2014; Elsheikha et al., 2016; Cavalera et al., 2018). The life-cycle of feline lungworms is similar to other species of gastropod-borne metastrongyloids, in which L1s are passed in the host faeces and reach the infective L3 stage in snail intermediate hosts (Anderson, 2000). Cats acquire the lungworm infection through the ingestion of intermediate or paratenic hosts harbouring infective L3s

* Corresponding author.
E-mail address: domenico.otranto@uniba.it (D. Otranto).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijpara.2018.12.008
0020-7519/

(Anderson, 2000; Colella et al., 2019). In the last decade, the role of gastropods as intermediate hosts has been widely studied in order to understand the epidemiology and the maintenance of metastrongyloid infection in the environment (Giannelli et al., 2016). *Troglostrongylus brevior* and *A. abstrusus* may simultaneously develop in the same mollusc species (i.e. *Cornu aspersum*), within approximately 11 days p.i., under laboratory conditions (Giannelli et al., 2014). Alternative transmission patterns, such as the release of L3s in the environment (Giannelli et al., 2015a) and the subsequent infection of other intermediate hosts, also known as intermediesis (Colella et al., 2015), have been investigated as further ways to maintain the parasite in the intermediate host population, therefore increasing the possibility to infect definitive or paratenic hosts. Anthelmintic products (e.g., Advocate®, Broadline®, Profender®) have been shown to be effective for the treatment of *A. abstrusus* (Traversa et al., 2009a,b; Crisi et al., 2017; Giannelli et al., 2017) and *T. brevior* (Crisi et al., 2017; Giannelli et al., 2017) infections in controlled studies and in clinical case reports (Pennisi et al., 2015). For example, 10% imidacloprid/1% moxidectin (Advocate®, Bayer) spot-on formulation has shown 100% efficacy in the treatment of feline aelurostrongylosis (Traversa et al., 2009a). A single topical administration of a combination of 8.3% fipronil, 10% (*S*)-methoprene, 0.4% eprinomectin and 8.3% praziquantel (Broadline®, Boehringer Ingelheim) was efficacious for the treatment of *A. abstrusus* and *T. brevior* infection under experimental and field conditions (Knaus et al., 2014; Rehbein et al., 2014; Giannelli et al., 2015b, 2017). The efficacy of tested drugs has been evaluated by comparing the faecal output of L1s measured pre-treatment and at a selected day during the follow-up (e.g., 28 ± 2 days). However, the shedding of feline lungworm L1s and their ability to infect and develop to L3s in the snail intermediate hosts after administration of anthelminthic products have not been previously investigated.

Hence, this study aims to (i) compare the efficacy of two different treatment products (i.e., Advocate® and Broadline®) against feline lungworm infection, (ii) determine the number of days post treatment until L1s are released in faeces and (iii) to evaluate the ability of L1s released with faeces of treated animals to infect and moult in the snail intermediate hosts.

2. Materials and methods

2.1. Snail maintenance

Twenty months old individual *C. aspersum* ($n = 600$) were purchased from a commercial provider of snails for human consumption and employed in this study. The gastropods were raised under laboratory conditions as previously described in Giannelli et al. (2014). To evaluate the absence of any natural infection by nematode larvae, the artificial digestion of 30 randomly selected snails and of all specimens that died naturally during the pre-infection period ($n = 10$) was performed.

2.2. Enrolment

The examination of cats was conducted with regard for animal welfare and the protocol was approved by the ethical committee of the Department of Veterinary Medicine at the University of Bari, Italy (Prot. Uniba 9/18). From November 2017 to September 2018, 384 client owned cats from two municipalities in Italy (i.e., Bari and Messina) were examined for lungworm infection. Individual cat data (i.e., age, gender, access to the outdoor environment, weight) and presence/absence of respiratory signs (i.e., sneezing, coughing, dyspnoea and nasal discharge) were recorded. Faecal samples were collected and analysed for the diagnosis of lungworms using a qualitative Baermann-Wetzel technique (Ministry of Agriculture, 1986). When present, larvae found in the sediment were morphologically identified using morphometric keys (Gerichter, 1949; Anderson, 2000; Brianti et al., 2014; Giannelli et al., 2014; Colella et al., 2017). Overall 36 cats which scored positive for lungworms, i.e., 18 positive for *A. abstrusus* and 18 positive for *T. brevior* infection, were recruited in the study following the owners' consent. All the enrolled animals were older than 9 weeks and weighed more than 1 kg.

2.3. Pre-treatment evaluation

For 3 days prior to treatment, faecal samples of enrolled cats were collected and then subjected to a quantitative Baermann technique to estimate the average numbers of L1s shed in the faeces. Briefly, 5 g of faeces were placed in double-layered gauze and secured with a wire; the sample was settled into a Baermann funnel which was filled with tap water, and examined after 24 h. The liquid was poured into a 50 ml tube and centrifuged at 600*g* for 5 min; the supernatant was removed to obtain a 5 ml sediment. Three aliquots of 50 μl each were placed on a microscope slide for examination and larvae were counted. The average number of larvae from the three aliquots was used to calculate the total L1s as: [average number of L1s in three aliquots: 50 μl = X:5000 μl], where X is the unknown total number of L1s in the sediment. The ratio of the total number of L1s and the grams of faeces (5 g) were used to express the number of larvae per gram (LPG) of faeces. Two snails were infected, each with doses of 100 L1s isolated during the 3 days, as previously described (Colella et al., 2016). After 28 days, infected snails were digested in order to estimate the development of L3s in gastropods before the treatment of cats (Colella et al., 2016). Each snail was digested, the suspension microscopically examined and larvae morphologically identified, as previously described (Gerichter, 1949; Giannelli et al., 2014).

2.4. Treatment protocol

Positive cats were randomly allocated to four groups (i.e, A-D) according to lungworm infection and the type of treatment received. Groups A and C were composed of cats ($n = 10$ each) positive for *T. brevior* and *A. abstrusus,* respectively, and treated with Broadline® spot-on solution. Groups B and D consisted of cats ($n = 8$ each) positive for *T. brevior* and *A. abstrusus*, respectively, and treated with Advocate® spot-on solution. Treatment with the selected products was performed according to label instructions, applying a single dose directly onto the skin.

2.5. Post-treatment evaluation

In addition to the 3 days prior to treatment, positive cats were followed up every day after the treatment until the animals become negative at the Baermann examination (i.e. 3 days of absence of lungworm L1s in the faeces) (Pennisi et al., 2015). At each day post treatment, the number of LPG of faeces was determined, and one/two snails (according to the larval availability) per day were infected with 100 L1s. After 28 days the infected snails were digested in order to estimate the rate of L3 development (Colella et al., 2015). The number of experimentally infected snails positive for L3s during the post-treatment period was calculated for each group. Furthermore, the morphology and the vitality of L3s recovered after the artificial digestion were evaluated. Larvae were considered dead when no movement was observed under a light microscope (Leica®, DL MB2) for up to 10 s, or when degeneration of larval internal organs occurred. Measurements (in micrometres) of L3s of *T. brevior* and *A. abstrusus* ($n = 20$ for each parasite species, randomly chosen) isolated after the artificial

digestion were also performed. Finally, treated animals were followed-up at 28 ± 2 days by faecal examination and, when positive, further treated.

2.6. Molecular analyses

DNA from larvae collected from faeces of infected and enrolled cats was extracted using the DNeasy Blood & Tissue Kit (Qiagen, GmbH, Hilden, Germany), following the manufacturer's instructions. All samples were analysed by a duplex-PCR for the detection of *A. abstrusus* and *T. brevior* (Annoscia et al., 2014) as well as by the amplification of the 18S rRNA gene (Patterson-Kane et al., 2009) and sequenced. Sequences were compared with those available in the GenBank™ database by using the Basic Local Alignment Search Tool (BLAST).

2.7. Data analysis

The daily larval output of lungworm-infected cats was expressed as normal logarithmic values [Log (LPG + 1)] for the calculation of geometric means for each treatment group and days post treatment. The efficacy of the treatments in the reduction of larval shedding was calculated using Abbot's formula: Efficacy (%) = 100[(*C* − *T*)/*C*], where *C* is the geometric mean of larvae in the pre-treatment and *T* is the geometric mean 28 days (±2) after the administration of the products. The geometric mean was calculated by averaging the log-counts (*x* + 1) of the single larval counts, taking the anti-logarithm and then subtracting 1. The difference between the number of days post treatment necessary for the infected cats to become negative according to species and treatment was statistically analysed using One-way ANOVA. Differences between the number of snails that became infected in the post treatment period according to species and group were investigated by Pearson's chi-square test (χ^2). A value of $P < 0.05$ was considered statistically significant. The statistical analyses were performed using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego California, USA).

3. Results

3.1. Prevalence of lungworm infection

Out of 384 animals analysed (i.e., 210 males and 174 females), 54 (14.1%) were positive for lungworms. Of the 54 infected cats, 24 (44.4%) and 28 (51.9%) scored positive for *T. brevior* and *A. abstrusus*, respectively, while two (3.7%) were infected by both species. Of the positive animals above, 36 were enrolled in this study and the remaining 18 were not recruited due to a lack of compliance from their owners ($n = 8$), the small number of larvae released in the faeces (i.e. less than 100 L1) ($n = 5$) or because they suddenly died before being treated ($n = 5$). In particular, all the five deceased cats (i.e., two positive to *T. brevior* and three to *A. abstrusus*) displayed a severe respiratory syndrome before the fatal outcome. *Troglostrongylus brevior*-infected cats that died before the end of the observational period were paediatric (i.e. less than 6 months) whereas those positive to *A. abstrusus* were young individuals (i.e. 6–24 months) (Hoyumpa Vogt et al., 2010). The post-mortem examination was performed only for a 1-year-old European shorthair cat infected by *A. abstrusus*. Lungs presented white-greyish irregularly shaped spots of consolidation interspersed with dark red and hyperaemic areas, surrounded by pulmonary emphysema.

3.2. Pre- and post-treatment larval counts

The analysis of the faeces collected during the pre-treatment period showed a geometric mean LPG of faeces of 233.3 (range 64.9–4206) in group A (*T. brevior*/Broadline®), 197.8 (range 13.7–5750) in B (*T. brevior*/Advocate®), 40.8 (range 10–300) in C (*A. abstrusus*/Broadline®) and 69.1 (range 10–5900) in D (*A. abstrusus*/Advocate®). The mean number of days post treatment necessary for the infected cats to become negative was 7.9 ± 1.2 (range 5–9) in group A (*T. brevior*/Broadline®), 7.8 ± 1.9 (range 5–11) in B (*T. brevior*/Advocate®), 6.9 ± 1.6 (range 5–9) in C (*A. abstrusus*/Broadline®) and 8.9 ± 2.0 (range 7–12) in D (*A. abstrusus*/Advocate®). One-way ANOVA analysis showed no statistically significant difference between the number of days post treatment necessary for the infected cats to become negative according to species and treatment ($P = 0.12$). The mean post-treatment LPG of faeces (log transformed) for groups A-D is shown in Fig. 1. All cats tested negative for lungworm larvae at 28 ± 2 days post treatment after a single drug administration. The overall efficacy with Broadline® and, similarly, with Advocate®, was 100% for *A. abstrusus* and *T. brevior*.

3.3. Pre-treatment infectivity of L1s

All specimens of *C. aspersum* ($n = 30$) artificially digested before the infection, as well as those that died during the pre-infection period ($n = 10$), were nematode-free. Out of 72 *C. aspersum* digested 28 days after infection with L1s ($n = 100$ per snail) of *A. abstrusus* or *T. brevior* obtained from faeces prior to treatment, 66 (91.7%) were positive for *A. abstrusus* and *T. brevior* L3s. Of the six snails in which the L1s did not develop, five and one had been infected with *T. brevior* and *A. abstrusus*, respectively. The mean number of L3s per snail infected prior to treatment is reported in Table 1.

3.4. Post-treatment infectivity of L1s

A total of 183 snails was experimentally infected with L1s of *A. abstrusus* and *T. brevior* collected from faeces in the post-treatment period and, more specifically, 60 in group A (*T. brevior*/Broadline®), 37 in B (*T. brevior*/Advocate®), 38 in C (*A. abstrusus*/Broadline®) and 48 in D (*A. abstrusus*/Advocate®). Following artificial digestion, L3s were found in 160 out of 183 (87.4%) experimentally infected snails. In particular, there were 51 (85%) positive gastropods in group A (*T. brevior*/Broadline®), 32 (86.5%) in B (*T. brevior*/Advocate®), 34 (89.5%) in C (*A. abstrusus*/Broadline®) and 43 (89.6%) in D (*A. abstrusus*/Advocate®). A total of 1524 L3s was detected in positive gastropods experimentally infected with post-treatment L1s and the mean number of L3s found 7 days post treatment is shown in Table 1. All larvae of either *A. abstrusus* or *T. brevior* recovered in the snails infected with L1s excreted post-treatment were motile and morphologically identified as potentially infective L3s since they had lost the external sheaths of the previous moulting (Gerichter, 1949; Giannelli et al., 2014). L3s ($n = 20$) measured 435.5 ± 27.2 µm (i.e., *T. brevior*) and 592.6 ± 30.3 µm (i.e., *A. abstrusus*). No statistically significant differences in the number of snails that became infected during the post treatment period have been found between Groups A and B ($\chi^2 = 0.041$, $P = 0.84$) nor between Groups C and D ($\chi^2 = 0.0003$, $P = 0.99$).

3.5. Molecular identification

The morphological identification of the L1s collected from faeces of infected cats was confirmed by duplex-PCR. The comparison of rDNA nucleotide sequences displayed 100% nucleotide identity

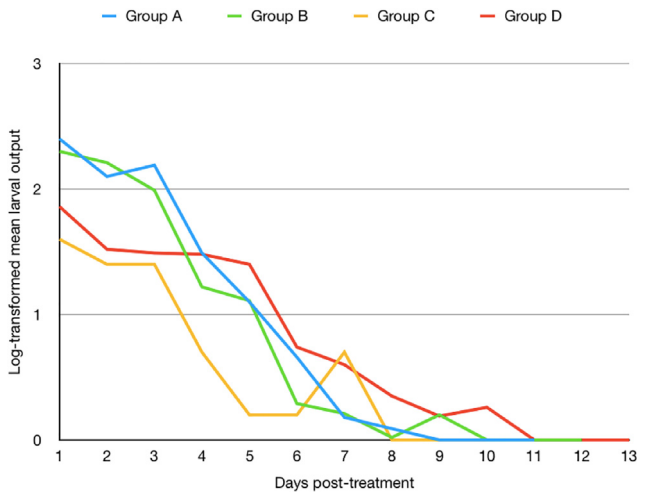

Fig. 1. Log-transformed mean post-treatment larval output per gram of faeces of animals belonging to groups A and C (i.e., positive to *Troglostrongylus brevior* and *Aelurostrongylus abstrusus*, respectively, and treated with Broadline®) and groups B and D (i.e., positive to *T. brevior* and *A. abstrusus*, respectively, and treated with Advocate®).

with those of *A. abstrusus* and *T. brevior* in GenBank (*A. abstrusus*, AJ920366 and *T. brevior*, JX290562).

4. Discussion

Cats successfully treated for lungworm infection with two endectocide formulations (i.e., Broadline® and Advocate®) shed live L1s which were able to infect intermediate hosts during the post-treatment period. Although enrolled cats were all negative for L1s at 28 ± 2 dpt by Baermann examination, faecal larval output reached negligible levels only after a minimum of 6.9 ± 1.6 (*A. abstrusus*/Broadline®) up to 8.9 ± 2.0 dpt (*A. abstrusus*/Advocate®). In this study, a single administration of Advocate® spot-on solution has been used for the first time in the off-label treatment of eight cats positive for *T. brevior* (i.e., group B) and was efficacious in stopping larval shedding. Information on the efficacy of this spot-on solution containing 1% moxidectin in the off-label treatment of *T. brevior* infection is scant and controversial. A single administration of Advocate® assured complete recovery in two kittens infected by *T. brevior* (Crisi et al., 2017), but failed to cure another one (Brianti et al., 2012). In addition, although this product stopped larval shedding in a 4-month-old patient, clinical, radiographic and echocardiographic signs of bronchopneumonia and pulmonary hypertension still persisted after several follow-ups (Crisi et al., 2015).

After administration of Broadline® or Advocate®, L1s released in the faeces displayed unchanged ability to parasitize and moult in the intermediate host, reaching the L3 stage, potentially infective for domestic cats. Indeed, L3s of *T. brevior* and *A. abstrusus* were found in 160 (87.4%) of the 183 experimentally infected snails. The mean number of L3s recovered from snails infected with L1s excreted during the first 7 days post-treatment varied considerably in all groups (Table 1). This fluctuation in the number of L3s from each specimen confirms the existence of an extreme variability of larval development in the intermediate hosts (Lange et al., 2018).

Free-roaming pet cats have an estimated home range (defined as the familiar space used by the animal to feed, mate or rest safely) (Powell and Mitchell, 2012) ranging from 0.002 km^2 to 0.2 km^2 (Hall et al., 2016). Therefore, the shedding of L1s from treated cats may contribute to the establishment and the dispersion of lungworms, eventually favouring the infection of new susceptible cats as well as the re-infection of treated animals roaming in the same home range. Noteworthy, once released by the definitive hosts, lungworm L1s persist in the environment (Gökpinar and Yildiz, 2010; Ramos et al., 2013). The survival of *T. brevior* larvae in the faeces is temperature-dependent, reaching 49 days at a controlled temperature of 4 °C and 35 days in the outdoor environment (i.e., mean temperature 14 ± 3.1 °C) (Ramos et al., 2013). Similarly, *A. abstrusus* L1s continue to live in faeces for 45 days at room temperature and 60 days at 4 °C (Gökpinar and Yildiz, 2010). In this context, cats with outdoor lifestyles have more opportunities to re-acquire lungworm infection by ingesting infected molluscs and/or paratenic hosts (Barutzki and Schaper, 2013; Beugnet et al., 2014; Colella et al., 2019).

The number of snails that became infected in the post-treatment period was not affected by the formulation employed (i.e., Broadline® and Advocate®). Overall, only 23 (12.5%) out of 183 infected snails were negative for L3s after the artificial digestion. The failure of L1 development in gastropods in the post-treatment period may be due to an effect of 0.4% eprinomectin (Broadline®) and 1% moxidectin (Advocate®) exerted against this larval stage (Knaus et al., 2014). Nonetheless, considering the presence of uninfected specimens among snails infected with pre-treatment L1s of *A. abstrusus* or *T. brevior*, snail immune responsiveness might halt larval development (Lange et al., 2017). For instance, moulting rates of 83.3% of *Crenosoma vulpis* (Colella et al., 2016) and of 96.7% of *Angiostrongylus chabaudi* (Colella et al., 2017) were recorded in previous studies in experimentally infected *C. aspersum*.

Data herein presented indicate that post-treatment shedding of L1s contributes to the infection of gastropods, potentially broadening the number of intermediate hosts available to the definitive hosts. Hence, recommendations in the post-treatment period should include keeping cats indoors for approximately 10 days

Table 1
Mean number and S.D. of L3s found in snails infected with L1s (n = 100) of *Troglostrongylus brevior* (Groups A and B) and *Aelurostrongylus abstrusus* (Groups C and D) shed in the faeces of cats in the pre-treatment and in the 7 days post-treatment (dpt) period.

	Pre-treatment (pos./infected snails)	1st dpt (pos./infected snails)	2nd dpt (pos./infected snails)	3rd dpt (pos./infected snails)	4th dpt (pos./infected snails)	5th dpt (pos./infected snails)	6th dpt (pos./infected snails)	7th dpt (pos./infected snails)
Group A (*T. brevior*/Broadline®)	12.1 ± 17.2 (15/20)	5.5 ± 5.2 (19/20)	9.6 ± 6.9 (12/16)	5.6 ± 5.7 (8/10)	6.3 ± 3.5 (8/8)	15.8 ± 10.5 (4/4)	– (0/2)	–
Group B (*T. brevior*/Advocate®)	5.6 ± 4.4 (16/16)	6.5 ± 3.6 (15/15)	8.6 ± 4.3 (8/10)	8.7 ± 3.4 (6/7)	7.5 ± 2.1 (2/4)	4 (1/1)	–	–
Group C (*A. abstrusus*/Broadline®)	33.2 ± 11.4 (20/20)	12.4 ± 8.6 (10/10)	6.9 ± 5.5 (12/14)	1.0 ± 0.0 (4/6)	4.8 ± 2.6 (4/4)	7.0 ± 1.4 (2/2)	3.5 ± 0.7 (2/2)	–
Group D (*A. abstrusus*/Advocate®)	28.3 ± 12.4 (15/16)	22.0 ± 21.6 (8/8)	17.8 ± 10.4 (14/14)	7.7 ± 8.1 (9/12)	12.6 ± 14.1 (5/6)	29.0 ± 1.4 (2/2)	16.7 ± 11.5 (3/4)	7.5 ± 3.5 (2/2)

pos., positive.

after drug administration in order to avoid environmental contamination and infection of snails.

Acknowledgements

The authors would like to thank Giulia Schino (DVM, Clinica Veterinaria Battelli) and all the cats' owners for their valuable collaboration.

References

Anderson, R.C., 2000. The superfamily Metastrongyloidea. In: Anderson, R.C. (Ed.), Nematode Parasites of Vertebrates. Their Development and Transmission. CABI, Wallingford, UK, pp. 129–229.

Annoscia, G., Latrofa, M.S., Campbell, B.E., Giannelli, A., Ramos, R.A., Dantas-Torres, F., Brianti, E., Otranto, D., 2014. Simultaneous detection of the feline lungworm *Troglostrongylus brevior* and *Aelurostrongylus abstrusus* by a newly developed duplex-PCR. Vet. Parasitol. 199, 172–178.

Barutzki, D., Schaper, R., 2013. Occurrence and regional distribution of *Aelurostrongylus abstrusus* in cats in Germany. Parasitol. Res. 112, 855–861.

Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., Halos, L., Joachim, A., Losson, B., Miró, G., Otranto, D., Renaud, M., Rinaldi, L., 2014. Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit. Vectors 7, 291.

Brianti, E., Gaglio, G., Giannetto, S., Annoscia, G., Latrofa, M.S., Dantas-Torres, F., Traversa, D., Otranto, D., 2012. *Troglostrongylus brevior* and *Troglostrongylus subcrenatus* (Strongylida: Crenosomatidae) as agents of broncho-pulmonary infestation in domestic cats. Parasit. Vectors 5, 178.

Brianti, E., Giannetto, S., Dantas-Torres, F., Otranto, D., 2014. Lungworms of the genus *Troglostrongylus* (Strongylida: Crenosomatidae): neglected parasites for domestic cats. Vet. Parasitol. 202, 104–112.

Cavalera, M.A., Iatta, R., Colella, V., Dantas-Torres, F., Corsaro, A., Brianti, E., Otranto, D., 2018. *Troglostrongylus brevior*: a feline lungworm of paediatric concern. Vet. Parasitol. 253, 8–11.

Colella, V., Giannelli, A., Brianti, E., Ramos, R.A., Cantacessi, C., Dantas-Torres, F., Otranto, D., 2015. Feline lungworms unlock a novel mode of parasite transmission. Sci. Rep. 5, 13105.

Colella, V., Knaus, M., Lai, O., Cantile, C., Abramo, F., Rehbein, S., Otranto, D., 2019. Mice as paratenic hosts of *Aelurostrongylus abstrusus*. Parasit. Vectors 12, 49.

Colella, V., Cavalera, M.A., Deak, G., Tarallo, V.D., Gherman, C.M., Mihalca, A.D., Otranto, D., 2017. Larval development of *Angiostrongylus chabaudi*, the causative agent of feline angiostrongylosis, in the snail *Cornu aspersum*. Parasitology 144, 1922–1930.

Colella, V., Mutafchiev, Y., Cavalera, M.A., Giannelli, A., Lia, R.P., Dantas-Torres, F., Otranto, D., 2016. Development of *Crenosoma vulpis* in the common garden snail *Cornu aspersum*: implications for epidemiological studies. Parasit. Vectors 9, 208.

Crisi, P.E., Aste, G., Traversa, D., Di Cesare, A., Febo, E., Vignoli, M., Santori, D., Luciani, A., Boari, A., 2017. Single and mixed feline lungworm infections: clinical, radiographic and therapeutic features of 26 cases (2013–2015). J. Feline Med. Surg. 19, 1017–1029.

Crisi, P.E., Traversa, D., Di Cesare, A., Luciani, A., Civitella, C., Santori, D., Boari, A., 2015. Irreversible pulmonary hypertension associated with *Troglostrongylus brevior* infection in a kitten. Res. Vet. Sci. 102, 223–227.

Elsheikha, H.M., Schnyder, M., Traversa, D., Di Cesare, A., Wright, I., Lacher, D.W., 2016. Updates on feline aelurostrongylosis and research priorities for the next decade. Parasit. Vectors 9, 389.

Gerichter, C.B., 1949. Studies on the nematodes parasitic in the lungs of Felidae in Palestine. Parasitology 39, 251–262.

Giannelli, A., Cantacessi, C., Colella, V., Dantas-Torres, F., Otranto, D., 2016. Gastropod-borne helminths: a look at the snail-parasite interplay. Trends Parasitol. 32, 255–264.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., René-Martellet, M., Papadopoulos, E., Farkas, R., Napoli, E., Brianti, E., Tamponi, C., Varcasia, A., Margarida, Alho A., Madeira de Carvalho, L., Cardoso, L., Maia, C., Mircean, V., Mihalca, A.D., Miró, G., Schnyder, M., Cantacessi, C., Colella, V., Cavalera, M.A., Latrofa, M.S., Annoscia, G., Knaus, M., Halos, L., Beugnet, F., Otranto, D., 2017. Lungworms and gastrointestinal parasites of domestic cats: a European perspective. Int. J. Parasitol. 47, 517–528.

Giannelli, A., Colella, V., Abramo, F., Ramos, R.A., Falsone, L., Brianti, E., Varcasia, A., Dantas-Torres, F., Knaus, M., Fox, M.T., Otranto, D., 2015a. Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways. PLoS Negl. Trop. Dis. 9, e0003722.

Giannelli, A., Brianti, E., Varcasia, A., Colella, V., Tamponi, C., Di Paola, G., Knaus, M., Halos, H., Beugnet, F., Otranto, D., 2015b. Efficacy of Broadline® spot-on against *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* lungworms in naturally infected cats from Italy. Vet. Parasitol. 209, 273–277.

Giannelli, A., Ramos, R.A., Annoscia, G., Di Cesare, A., Colella, V., Brianti, E., Dantas-Torres, F., Mutafchiev, Y., Otranto, D., 2014. Development of the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in *Helix aspersa* snails. Parasitology 141, 563–569.

Gökpinar, S., Yildiz, K., 2010. The effect of different temperatures on viability of *Aelurostrongylus abstrusus* first stage larvae in faeces of cats. Turkiye Parazitol Derg. 34, 102–105.

Hall, C.M., Bryant, K.A., Fontaine, J.B., Calver, M.C., 2016. Do collar-mounted predation deterrents restrict wandering in pet domestic cats? Appl. Anim. Behav. Sci. 176, 96–104.

Hoyumpa Vogt, A., Rodan, I., Brown, M., Brown, S., Buffington, C.A., Larue Forman, M. J., Neilson, J., Sparkes, A., 2010. AAFP-AAHA: feline life stage guidelines. J. Feline Med. Surg. 12, 43–54.

Knaus, M., Chester, S.T., Rosentel, J., Kühnert, A., Rehbein, S., 2014. Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against larval and adult stages of the cat lungworm, *Aelurostrongylus abstrusus*. Vet. Parasitol. 202, 64–68.

Lange, M.K., Penagos-Tabares, F., Hirzmann, J., Failing, K., Schaper, R., Van Bourgonie, Y.R., Backeljau, T., Hermosilla, C., Taubert, A., 2018. Prevalence of *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Crenosoma vulpis* larvae in native slug populations in Germany. Vet. Parasitol. 254, 120–130.

Lange, M.K., Penagos-Tabares, F., Muñoz-Caro, T., Gärtner, U., Mejer, H., Schaper, R., Hermosilla, C., Taubert, A., 2017. Gastropod-derived haemocyte extracellular traps entrap metastrongyloid larval stages of *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*. Parasit. Vectors 10, 50.

Ministry of Agriculture, 1986. Fisheries and food. Manual of Veterinary Parasitological Laboratory Techniques. HMSO, London, UK.

Patterson-Kane, J.C., Gibbons, L.M., Jefferies, R., Morgan, E.R., Wenzlow, N., Redrobe, S.P., 2009. Pneumonia from *Angiostrongylus vasorum* infection in a red panda (*Ailurus fulgens fulgens*). J. Vet. Diagn. Invest. 21, 270–273.

Pennisi, M.G., Hartmann, K., Addie, D.D., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Horzinek, M.C., Hosie, M.J., Lloret, A., Lutz, H., Marsilio, F., Radford, A.D., Thiry, E., Truyen, U., Möstl, K., European Advisory Board on Cat Diseases, 2015. Lungworm disease in cats: ABCD guidelines on prevention and management. J. Feline Med. Surg. 17, 626–636.

Powell, R., Mitchell, M., 2012. What is a home range? J. Mammal. 93, 948–958.

Ramos, R.A., Giannelli, A., Dantas-Torres, F., Brianti, E., Otranto, D., 2013. Survival of first-stage larvae of the cat lungworm *Troglostrongylus brevior* (Strongylida: Crenosomatidae) under different conditions. Exp. Parasitol. 135, 570–572.

Rehbein, S., Capári, B., Duscher, G., Keidane, D., Kirkova, Z., Petkevicius, S., Rapti, D., Wagner, A., Wagner, T., Chester, S.T., Rosentel, J., Tielemans, E., Visser, M., Winter, R., Kley, K., Knaus, M., 2014. Efficacy against nematode and cestode infections and safety of a novel topical fipronil, (S)-methoprene, eprinomectin and praziquantel combination product in domestic cats under field conditions in Europe. Vet. Parasitol. 202, 10–17.

Traversa, D., Di Cesare, A., Milillo, P., Lohr, B., Iorio, R., Pampurini, F., Schaper, R., Paoletti, B., Heine, J., 2009a. Efficacy and safety of Imidacloprid 10 %/Moxidectin 1 % spot-on formulation in the treatment of feline aelurostrongylosis. Parasitol. Res. 105, S55–S62.

Traversa, D., Milillo, P., Di Cesare, A., Lohr, B., Iorio, R., Pampurini, F., Schaper, R., Bartolini, R., Heine, J., 2009b. Efficacy and safety of emodepside 2.1%/praziquantel 8.6% spot-on formulation in the treatment of feline aelurostrongylosis. Parasitol. Res. 105, S83–S89.