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Efficacy of a topical combination of eprinomectin, praziquantel, fipronil and (S)-methoprene against developing and adult *Troglostrongylus brevior* lungworms (Nematoda, Crenosomatidae) in cats

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ABSTRACT

The efficacy of the eprinomectin, praziquantel, fipronil and (S)-methoprene combination parasiticide Broadline® (Boehringer Ingelheim Animal Health) was evaluated against developing larval and adult stages of *Troglostrongylus brevior*, a metastrongyloid pulmonary nematode which is reported to parasitize domestic cats in southern Europe with increasing frequency.

Twenty four purpose-bred cats were experimentally infected with 100 third-stage *T. brevior* larvae each and randomly allocated to either remain untreated (control) or to be treated with the combination product when *T. brevior* were developing larval (6 days post inoculation, dpi) or adult nematodes (28 dpi) (eight cats per group). Treatments were administered topically at the minimum label dose of 0.12 mL/kg. Fecal samples of the cats were examined to confirm the presence of patent (adult) nematode infections prior to treatment at 28 dpi and to monitor the larval excretion. At necropsy (49 dpi), the weight of the pulmonary lymph nodes and lungs were determined, and *T. brevior* lungworms were recovered and counted.

All control animals and cats to be treated at 28 dpi excreted *T. brevior* larvae 24 dpi and 26 dpi while no larvae were excreted by the cats treated at 6 dpi. Following treatment at 28 dpi, *T. brevior* larval excretion decreased immediatetly and ceased prior to necropsy. Nematode counts demonstrated that treatment with the combination product was 100 % efficacious against both developing larval and adult *T. brevior*: no lungworms were recovered from any treated cat while all control animals harbored *T. brevior* (range, 6–52) ($p < 0.001$).

No treatment-related health problems or any other clinical signs were observed in the cats. However, significantly higher absolute and relative (organ weight to body weight ratio) pulmonary lymph node weights of the control animals compared with the treated cats at 6 dpi ($p < 0.001$ and $p < 0.001$, respectively) and at 28 dpi ($p = 0.003$ and $p = 0.019$, respectively) indicated the pathology of the *T. brevior* infection.

In conclusion, the combination product was demonstrated to be 100 % efficacious against developing larval and adult *T. brevior*. Furthermore, it was demonstrated that indicators of impaired respiratory and immune systems resultant from *T. brevior* infection can be prevented with an efficacious treatment when administered during the pre-patent period of infection or are improving substantially within three weeks of treatment of cats harboring adult lungworms.

1. Introduction

Until being recently reported in domestic cats from Ibiza and Sicily in the Mediterranean (Jefferies et al., 2010; Brianti et al., 2012) based on the identification of first-stage larvae or adult nematodes, respectively, *Troglostrongylus* lungworms were known from Europe only as occasional parasites of wildcats, *Felis s. silvestris*, from mainland Italy, Bulgaria and Slovakia (Paggi, 1959; Genov, 1971; Ânčev and Genov, 1978; Mituch et al., 1988; Gaglio et al., 2010).

Since then, an increasing number of *T. brevior* cases in domestic cats have been reported, mainly based on morphological and/or molecular identification of larvae extracted from cat feces, including records from Spain (Giannelli et al., 2017), Italy (Brianti et al., 2013; Annoscia et al., 2014; Di Cesare et al., 2014, 2015a, 2015b; Giannelli et al., 2014, 2015, 2017; Tamponi et al., 2014; Traversa et al., 2014, 2015, 2019; Crisi et al., 2015, 2017; Varcasia et al., 2015; Cavalera et al., 2018, 2019),

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Greece (Diakou et al., 2014, 2015), Bulgaria (Giannelli et al., 2017) and Cyprus (Diakou et al., 2017). Recently, a pair of *T. brevior* was isolated from a cat co-infected with *Aelurostrongylus abstrusus* in Albania (Knaus and Vokshi; unpublished), and the recovery of *T. brevior* larvae from the feces of one cat in Poland was reported (Szcepaniak et al., 2019). In addition, there were new records of *T. brevior* in wildcats from Italy (Beraldo et al., 2014; Falsone et al., 2014; Veronesi et al., 2016), Germany (Steeb, 2015) and Romania (Deak et al., 2017). Interestingly, Ibragimova and Rzajev (2017) recently reported the recovery of *T. brevior* from herding dogs and stray dogs in Azerbaijan. However, apart from listing *T. brevior* among 14 or 22 species of nematodes recovered from the two categories of dogs, respectively, no other information is provided and authors did not acknowledge their findings as first records of *Troglostrongylus* lungworms in canids.

The increasing number of records in domestic cats of *T. brevior* and the diagnosis of infection associated with clinical respiratory disease and even death in naturally infected very young cats (Brianti et al., 2012, 2013; Giannelli et al., 2014, 2017; Tamponi et al., 2014; Traversa et al., 2014, 2015, 2018; Crisi et al., 2015, 2017, 2018; Di Cesare et al., 2015b; Cavalera et al., 2018) stimulated the interest of practicing veterinarians and parasitologists in troglostrongylosis and highlighted the desire in an authorized treatment.

Broadline®, a broad-spectrum combination parasiticide containing the macrocyclic lactone eprinomectin, was previously demonstrated to be efficacious against *A. abstrusus* and *Eucoleus aerophilus* lungworm infections in cats (Knaus et al., 2014, 2015). The controlled study reported here was conducted to evaluate the therapeutic efficacy of a single topical treatment with this topical combination product at the minimum recommended dose against developing larval and adult *T. brevior* lungworms in cats.

2. Material and methods

The design of the study was in accordance with general recommendations of the VICH Guidelines 7 and 20 (Vercruysse et al., 2001, 2002) and the WAAVP guidelines for evaluating the efficacy of anthelmintics in dogs and cats (Jacobs et al., 1994). The study complied with VICH Guideline 9, entitled *Good Clinical Practice* and was a blinded study, i.e., all personnel involved in collecting efficacy data and making health observations were masked as to the treatment assignment of the animals.

2.1. Experimental animals and inoculation

The study included 24 (14 male, 10 female) healthy nematode-naïve, purpose-bred short-haired cats which were approximately five to six months of age and weighed between 2.23 and 3.58 kg at the time of allocation. The animals were housed individually throughout the study under identical conditions. All animals were handled with due regard to their welfare and in compliance with the company's Institutional Animal Care and Use Committee approvals and any applicable local regulation.

Each cat was inoculated orally once with approximately 100 infective third-stage *T. brevior* larvae which were extracted from experimentally infected *Helix aspersa* snails by peptic digestion. The isolate used was a recent field isolate as defined per VICH GL 7 (Vercruysse et al., 2001) which was obtained from a naturally infected cat from Italy.

2.2. Study design

The study employed a randomized block design with blocks of three animals formed on pre-treatment body weight. Within blocks, animals were allocated at random to one of three treatment groups: untreated (control) or to be treated with Broadline® (eprinomectin 0.4 % w/v, praziquantel 8.3 % w/v, fipronil 8.3 % w/v, (S)-methoprene 10 % w/v) once either 6 days post inoculation (dpi) or 28 dpi when *T. brevior* were developing larval nematodes or adult parasites (as confirmed by positive fecal *T. brevior* larval counts), respectively. Treatments were administered topically at the minimum label dose of 0.12 mL per kg body weight directly on the skin in the midline of the neck between the base of the skull and the shoulder blades in a single spot. All cats were observed hourly for four hours post-treatment and thereafter daily until the end of the study for adverse events and clinical signs.

All cats were weighed at 2 dpi for allocation to treatment groups and dose calculation of cats treated 6 dpi, and at 49 dpi, prior to necropsy; cats treated at 28 dpi were weighed prior to dosing for dose calculation.

2.3. Sample collection and examination

Fecal samples (10 g each) were collected from all cats 24 dpi and 26 dpi and twice weekly thereafter until study end and subjected to the Baermann-Wetzel migration technique to establish *T. brevior* first-stage larval counts.

After determination of body weight, cats were necropsied 49 dpi. The heart-lung complex (including trachea) was removed, and the heart was separated. Thereafter, the pulmonary lymph nodes (Lymphonodi tracheobronchiales [dexter, sinister and medius] and Ln. pulmonaris) were separated from the lungs and their total weight as well as the weight of the lungs was determined.

For parasite recovery, the airways of the lungs were opened lengthwise using fine scissors and nematodes were recovered. The opened lungs were soaked in saline overnight to allow for the collection of potentially remaining nematodes. Isolated nematodes were confirmed to be *T. brevior* based on morphology (Gerichter, 1949) and enumerated.

2.4. Data analysis

T. brevior counts were transformed to the natural logarithm of (count + 1) for calculation of geometric means for each treatment group. Efficacies for the treated groups were determined by calculating the percent efficacy as 100x[(C–T)/C], where C was the geometric mean among untreated controls and T was the geometric mean among the treated animals. The log-counts of the treated groups were compared with the log-counts of the untreated control group. All comparisons used an F-test adjusted for the allocation blocks used to randomize the animals to the treatment groups, with the treatment groups listed as a fixed effect, and the allocation blocks listed as a random effect.

The lung, pulmonary lymph node, and body weights were compared among the treatment groups. To adjust lung and pulmonary lymph node weights for different animal sizes, the lungs:body weight ratio and pulmonary lymph nodes:body weight ratio were also compared. The ratios were expressed as lung or pulmonary lymph node weights per 100 g body weight. They were calculated by converting numerator and denominator to the same units, computing the ratio and then multiplying by 100. The lung, pulmonary lymph node and body weights were analyzed untransformed. So the analysis would be on differences, it was the logarithm of the lungs:body weight and pulmonary lymph nodes: body weight ratios that was analyzed. The treatment groups were compared using an F-test as described above.

The Mixed procedure in SAS Version 9.4 was used for the analyses, and all testing was two-sided at the significance level $\alpha = 0.05$.

3. Results

No adverse events or other health problems were observed after administration of the topical combination product and throughout the study. Daily observations did not reveal clinical abnormalities.

Animal details per treatment group, results of fecal examinations prior to administration of the treatment at 28 dpi and *T. brevior* lungworm counts are summarized in Table 1. Geometric mean fecal *T. brevior* larval counts over the course of the study are plotted in Fig. 1.

Twenty-four dpi and 26 dpi, all cats except those that were treated at

Table 1
Troglostrongylus brevior fecal larval and adult nematode counts of untreated controls and cats treated with Broadline® at 6 days post inoculation (dpi) (developing larval infections) or 28 dpi (adult infections), and percentage therapeutic efficacy based on parasite counts (n = 8 per group).

Treatment Group; Number and sex of cats included	GM[1] *Troglostrongylus brevior* larval per gram of feces counts (range)		GM *Troglostrongylus brevior* adult nematode counts (range), P-value[2] and % Efficacy[3]
	24 dpi[4]	26 dpi	49 dpi
Untreated (control); 5 male, 3 female	59.3 (9–281)	178.0 (26.3–480)	28 (6–52)
Treated, 6 dpi; 4 male, 4 female	0	0	0 P<0.001 100 %
Treated, 28 dpi; 5 male, 3 female	72.8 (10–555)	194.7 (45–462.5)	0 P<0.001 100 %

[1] GM = geometric mean, computed by subtracting 1 from the anti-logarithm of the mean of ln(count+1).
[2] Two-sided P-value from comparing nematode counts Controls and treated groups.
[3] %Efficacy = 100 x [(C–T)/C], where C is the GM of Controls and T is the GM of treated groups.
[4] dpi = days post inoculation.

6 dpi, excreted *T. brevior* larvae and thus the presence of adult (patent) nematode infections in these animals were confirmed. While no larvae were recovered from the feces of the cats treated at 6 dpi throughout the study, excretion of *T. brevior* larvae was demonstrated in all untreated (control) animals at each occasion. Following treatment of the cats with patent infections at 28 dpi, *T. brevior* larval counts declined by almost 95 % (p = 0.001) within five days and ceased completely prior to necropsy.

At necropsy 49 dpi, six to 52 adult *T. brevior* lungworms were recovered from the eight untreated (control) cats. Thus, the inoculation with approximately 100 third-stage *T. brevior* larvae produced adequate levels of infections in the sense of VICH GLs 7 and 20 (Vercruysse et al., 2001, 2002). Overall, 267 *T. brevior* lungworms were recovered as adult nematodes from the untreated (control) animals. Mean sex ratio of lungworms recovered was 1.65:1 for females versus males. No lungworms were recovered from any treated cat which demonstrated treatment to be 100 % efficacious against developing larval and adult *T. brevior* nematode infections.

Results of the analyses of lung, pulmonary lymph node, and body weights (absolute weights), and lungs:body weight ratio and pulmonary lymph nodes:body weight ratio (relative weights) are summarized in Table 2. Absolute and relative pulmonary lymph node weights were significantly lower for the cats treated at 6 dpi or 28 dpi than for the untreated (control) animals ($p \leq 0.003$ and $p \leq 0.019$, respectively) (Fig. 2). Cats treated at 6 dpi had lower absolute and relative pulmonary lymph node weights than the cats treated at 28 dpi ($p = 0.075$ and $p = 0.022$, respectively). Absolute and relative lung weights of the cats treated at 6 dpi or at 28 dpi and of the cats treated at 28 dpi or remained untreated (control) were similar ($p \geq 0.177$); however, absolute and relative lung weights of the cats treated at 6 dpi cats tended to be lower than those of untreated (control) animals ($p = 0.058$ and $p = 0.070$, respectively).

4. Discussion

The primary objective of the study was to determine the efficacy of Broadline® against pre-patent and patent infections with the pulmonary nematode *T. brevior* of cats in a controlled study. The results of this study demonstrate that this topical combination product when administered at the minimum recommended dose of 0.12 mL per kg body weight was

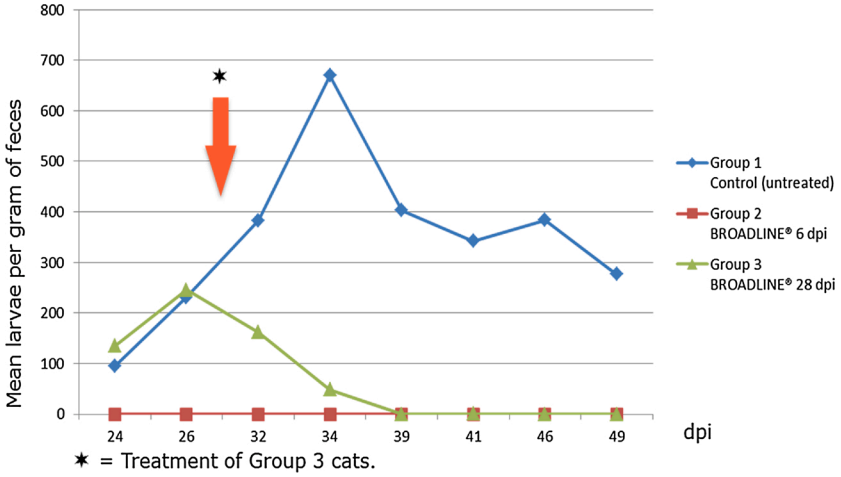

Fig. 1. Geometric mean fecal *Troglostrongylus brevior* larval counts per gram over the course of the study (dpi = days post inoculation).

Table 2
Analyses of absolute lung, pulmonary lymph node and body weights, and lungs: body weight ratio and pulmonary lymph nodes : body weight ratio (relative weights).

Parameter	Arithmetic Mean			P-values, pairwise comparisons[1]		
	Untreated (control)		Treated 28 dpi	Control *vs.* Treated 6 dpi	Control *vs.* Treated 28 dpi	Treated 6 dpi *vs* Treated 28 dpi
Lung weight (g)	56.18	44.34	48.01	0.058	0.177	0.532
Pulmonary lymph nodes weight (g)	2.11	0.78	1.25	<0.001	0.003	0.075
Body weight (kg)	3.16	3.20	3.17	0.765	0.949	0.815
Lungs : body weight ratio (g/100 g × 100)	1.74	1.34	1.46	0.070	0.199	0.552
Pulmonary lymph nodes : body weight ratio (g/100 g × 100)	0.065	0.023	0.038	<0.001	0.019	0.022

[1] Two-sided p-value comparing weights or weight ratios of the untreated and treated groups.

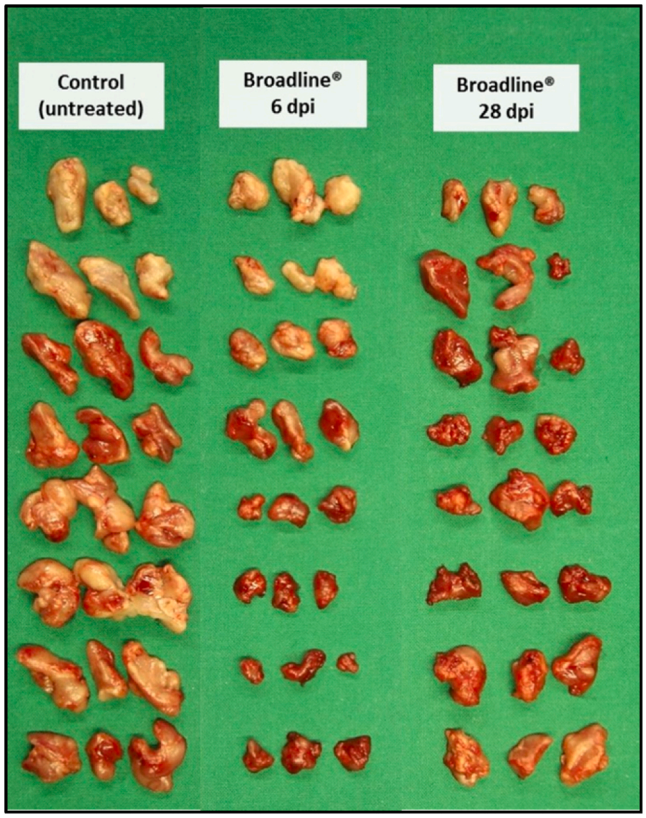

Fig. 2. Individual pulmonary lymph nodes arranged by treatment group.

100 % effective in eliminating developing and adult *T. brevior* infections from cats. Previously, the effect of treatment with this product against *T. brevior* lungworms was known only from the suppression of fecal larval excretion in naturally infected cats that were administered the label dose (Giannelli et al., 2015, 2017; Cavalera et al., 2019).

Although not yet studied, the development of *T. brevior* in the final host is likely to be similar to that of other members of the Crenosomatidae family of lungworms which, collectively, are ovoviviparus nematodes that reside as adult parasites in the larger airways of the lungs (tracheo-bronchial tract) of their final hosts (Anderson, 2000). Studies on the development in the definitive host of four species of *Crenosoma* (*C. mephitidis*, *C. petrowi*, *C. striatum*, *C. vulpis*) found similarly that, after infection with third-stage larvae, the third and fourth moults occurred approximately four and seven days, respectively, post inoculation and that the pre-patent periods ranged between 18 and 25 days (Anderson, 2000). The cats in the present study excreted *T. brevior* larvae in their feces 24 dpi, and Gerichter (1949) previously observed first shedding of *T. brevior* larvae 28 dpi in a suckling kitten. These findings support that the temporal development of *T. brevior* in the cat is likely similar to that of the *Crenosoma* species in their mammal hosts so that it can reasonably be assumed that the *T. brevior* were developing fourth-stage larval nematodes when treatment was administered to the cats 6 dpi in the present study.

The most obvious signs reported in clinically affected domestic cats naturally infected with *T. brevior* were sneezing, coughing, nasal discharge and dyspnoe (Jefferies et al., 2010; Traversa and Di Cesare, 2016; Crisi et al., 2017, 2018; Giannelli et al., 2017; Cavalera et al., 2018; Traversa et al., 2019). The cats in this study, however, were clinically unremarkable during the daily observations despite harbouring up to 52 *T. brevior* lungworms. Given that the majority of young cats which were reported as fatal cases of troglostrongylosis harboured only few lungworms (Brianti et al., 2012, 2013; Giannelli et al., 2014; Traversa et al., 2015, 2018), it can be assumed that in the course of *T. brevior* infections, similar with respiratory nematode infections in other mammals, secondary pulmonary infections with microbial pathogens play a role and/or other conditions which are prone to enhance the susceptibility of cats to infections in general and/or to compromise their immune status (e.g. underlying disease, overpopulation situations, insufficient care, undernourishment, stray cats) may affect the general health status and condition of the animals. In addition, it should be considered that larger surveys found that less than one third of the cats naturally infected with *T. brevior* exhibiting respiratory signs (Giannelli et al., 2017; Cavalera et al., 2018). The cats in the present study, despite belonging to the age class which has been reported to be at higher risk of troglostrongylosis (Cavalera et al., 2018), were fully vaccinated and well-cared for and therefore likely able to withstand the development of clinical signs following the experimental infection. The absence of obvious signs of respiratory abnormality in all 16 cats with patent infections in this study may suggest that, within the limits of this experiment including the size of the inoculum, *T. brevior* is relatively well adapted to domestic cats. Earlier experimental studies with *A. abstrusus* have shown that the development of clinical signs in cats were directly related to the number of infectious larvae used for inoculation and were especially pronounced in cats inoculated with 800 or more larvae and after handling of the cats (Hamilton, 1967). However, pathogenicity of *T. brevior* infection was clearly documented in the cats in this study by the enlargement of the pulmonary lymph nodes associated with higher weights of the lungs. Apart from physiological organ growth and differentiation of tissues within an organ, organ weight (size), to a great extent, is regulated in response to the organ's functional demand. In the wildcat, the most common lesions associated with *T. brevior* infection have been characterized as mild to severe chronic catarrhal bronchitis (Veronesi et al., 2016) which likely stimulated the enlargement of the lymph nodes which drain the affected lungs as peripheral lymphoid organs (activation following continuous exposure to *T. brevior*-derived and probably other antigens). Reactive lymph node enlargement that has been reported also in cats with experimentally induced *A. abstrusus* infection represents the morphological equivalent of an immunological response (Hamilton, 1966; Schnyder et al., 2014). Efficacious treatment with this topical combination product of cats during the pre-patent and patent phases of infection with *T. brevior* resulted in a reduction of antigen exposure of the immune system as reflected in significantly lower weights of the pulmonary lymph nodes in the treated cats compared with the control animals. So far, no studies have investigated the immunology of troglostrongylosis. However, acquired immunity may play a role as prevalence of *T. brevior* infection in naturally infected cats appears to be inversely related to the age of the cats (Tamponi et al., 2014; Giannelli et al., 2017; Cavalera et al., 2018).

In conclusion, the results of the present study demonstrated that Broadline® is a highly effective feline anthelmintic against developing larval and adult *T. brevior* lungworms and with that, the topical combination product has proven to be efficacious against the three most common pulmonary nematodes which infect domestic cats in Europe: the metastrongyloids *A. abstrusus* and *T. brevior*, and the trichuroid *E. aerophilus*. In addition, results of the present study demonstrated that effective treatment of cats infected with *T. brevior* lungworms is associated with beneficial effects on the pathology of troglostrongylosis and the health status of the cats.

Declaration of Competing Interest

The work reported herein was funded by Boehringer Ingelheim Animal Health of which all authors are current employees.

CRediT authorship contribution statement

Martin Knaus: Methodology, Investigation, Writing - original draft. **Martin Visser:** Investigation. **Sandra Mayr:** Investigation. **Steffen Rehbein:** Supervision, Writing - review & editing.

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Broadline® is a trademark of Boehringer Ingelheim Animal Health; all other marks are the property of their respective owners.

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