Veterinary Parasitology 209 (2015) 273–277

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short Communication

Efficacy of Broadline® spot-on against *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* lungworms in naturally infected cats from Italy☆

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ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 23 February 2015
Accepted 28 February 2015

Keywords:
Aelurostrongylus abstrusus
Troglostrongylus brevior
Lungworms
Broadline®
Cats
Treatment

ABSTRACT

The increasing reports of *Aelurostrongylus abstrusus* infection and the new information on *Troglostrongylus brevior* have spurred the interest of the scientific community towards the research of pharmaceutical compounds effective against both pathogens. A novel topical combination of fipronil, (*S*)-methoprene, eprinomectin and praziquantel (Broadline®, Merial) has been released for the treatment of a variety of feline parasitic infections. The present study reports the efficacy of this spot-on in treating cats naturally infected by feline lungworms. Client owned cats ($n = 191$) were enrolled from three geographical areas of Italy and faecal samples were examined by floatation and Baermann techniques. Twenty-three individuals were positive for L1 of *A. abstrusus* ($n = 18$) or *T. brevior* ($n = 3$) or for both species ($n = 2$) and they were topically treated with Broadline®. Seventeen of them were also concomitantly infected by other parasites. Four weeks after treatment, faecal samples were collected and examined to assess the efficacy of a single administration of the product. Based on lungworm larvae counts, the efficacy of the treatment was 90.5% or 100% for *A. abstrusus* or *T. brevior*, respectively. Cats released significantly lower amounts of lungworm larvae after treatment compared to pre-treatment ($p < 0.0001$). All but three cats were negative for other nematodes after treatment and all cats recovered from respiratory signs. Results of this study indicate that a single administration of the topical combination fipronil, (*S*)-methoprene, eprinomectin and praziquantel is effective and safe for the treatment of *A. abstrusus* and/or *T. brevior* infections in cats living under field conditions.

1. Introduction

Domestic cats may be infected by several endoparasites, which may cause a variety of clinical signs, ranging from digestive alterations to anaemia, and respiratory distress (Beugnet et al., 2014). Gastrointestinal helminths, protozoans and lungworms are accounted as the major

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http://dx.doi.org/10.1016/j.vetpar.2015.02.037

cause of parasitic diseases in cats across Europe (Beugnet et al., 2014). Some of these species (e.g., *Toxocara cati*, *Toxoplasma gondii*, *Giardia lamblia*) represent a risk for human beings (Traversa, 2012), whereas others (e.g., lungworms), though are not of zoonotic concern, may be highly pathogenic for cats and cause severe life-threatening conditions (Gerdin et al., 2011). In addition, some intestinal parasites may cause self-limited infections but their presence is not acceptable by owners. Consequently, the administration of an efficacious treatment against these pathogens, as well as the prevention of the infection, represents either an act of responsibility towards animal welfare and Public Health.

Among the lungworms infecting cats, *Aelurostrongylus abstrusus* (Strongylida, Angiostrongylidae) is the most prevalent species across Europe (Beugnet et al., 2014) and several field studies have provided data on the usefulness of anthelmintic treatments against this nematode (Traversa et al., 2009; Iannino et al., 2013). Nonetheless, the recent report of feline troglostrongylosis by *Troglostrongylus brevior* (Strongylida, Crenosomatidae), along with the little information available on its treatment and biology, has spurred the interest of the scientific community towards the research of pharmaceutical compounds effective against both pathogens (Brianti et al., 2012).

Recently, a novel topical combination of fipronil 8.3% (w/v), (*S*)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v) (Broadline®, Merial), hereinafter referred to as the product, has been registered for the treatment of a broad spectrum of parasites-causing infections in cats (e.g., *Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis*, *Ancylostoma tubaeforme*, *Dirofilaria immitis*, *Ctenocephalides felis*, *Ixodes ricinus*) (Rehbein et al., 2014). The main active ingredient, which is effective against nematodes (i.e., eprinomectin), belongs to the avermectin family and it affects pathogens by irreversibly opening the glutamate-gated chloride ion channels and inducing cell hyperpolarization (Wolstenholme, 2012). This product has been tested in domestic cats for safety and efficacy against natural nematode and tapeworm infections, and was demonstrated highly efficacious against ascarids (i.e., *T. cati* and *Toxascaris leonina*), hookworms (i.e., *A. tubaeforme* and *Ancylostoma braziliense*) and *Capillaria* (syn. *Eucoleus*) spp. (Rehbein et al., 2014). Moreover, in a single study conducted under experimental conditions, the product showed to be highly effective for the treatment of different developmental stages of *A. abstrusus* in cats (Knaus et al., 2014a). However, this formulation has never been tested in cats living under natural conditions, where multiple infections may occur, as well as in *T. brevior*-infected animals. Therefore, the aim of the present study was to assess the efficacy of Broadline® spot-on in cats naturally infected by *A. abstrusus* and/or *T. brevior*.

2. Material and methods

2.1. Prevalence of endoparasite infections in cats at the enrolment

A field trial study was conducted in three geographical sites of Italy, (i.e., Messina, Sassari and Bari), where the occurrence of feline lungworm infections had been previously reported (Brianti et al., 2012; Annoscia et al., 2014; Giannelli et al., 2014a; Tamponi et al., 2014). From May to September 2014, client owned cats, which had regular access to the outdoor environment, were clinically examined. Following the receipt of the owners' informed consent, anamnestic data (i.e., age, gender, clinical presentation and weight) were collected and faecal samples were examined for gastrointestinal parasites and lungworm nematodes. In particular, faeces (at least 10 g) were subjected to flotation with $ZnSO_4$ (specific gravity = 1.2) and quantitative Baermann technique, as described elsewhere (MAFF, 1986; Zajac and Conboy, 2012). Briefly, faecal samples were placed into Baermann funnels, filled with 50 ml warm tap water and, after 24 h, the liquid was collected into a tube and centrifuged at 2500 rpm ($600 \times g$) for 5 min. Finally, the supernatant was removed and the sediment was examined on a microscope slide. The whole number of larvae was counted and lungworms were identified at species level (Gerichter, 1949; Giannelli et al., 2014b), thus assessing the number of larvae per gram (Lpg) and the presence of co-infections. Parasites in the faeces were identified based on their morphology (Euzeby, 1981; MAFF, 1986).

2.2. Evaluation of treatment against lungworms

Cats positive for lungworm larvae were enrolled in the study if owners consented to treatment with Broadline® spot-on and to subsequent follow-up. The product was administered topically on the midline of the neck, between the base of the skull and the shoulder blades, in a single spot, as described in the label. Specifically, cats weighing 0.8–2.5 kg received 0.3 ml dose, while animals weighing 2.5–7.5 kg received a dose of 0.9 ml. The owners were requested to report any health problems or adverse events during the course of the study and animals were followed-up 28 ± 2 days after treatment, with clinical and faecal examinations performed as described above.

2.3. Data analysis

A. abstrusus and *T. brevior* Lpg were transformed into the natural logarithm (count +1) for calculation of geometric means for each species. The efficacy of the treatment was calculated as percentage, using the formula: efficacy (%) = $100[(C - T)/C]$, where C was the geometric mean of larvae before the treatment and T was the geometric mean following the administration of the product. The change in counts was analysed by modelling the logarithm of the counts +1 by a repeated measures analysis of variance, where the measurement time (pre-treatment versus post-treatment) was the repeated measurement within the individual animal. The mixed procedure in SAS® Version 9.1.3 was used for the analysis with measurement time defined, as the fixed effect as well as repeated measure, and animal defined as the subject. All analyses were two-sided at the significance level of $\alpha = 0.05$.

Table 1
Number and prevalence of cats infected by gastrointestinal parasites and lungworm nematodes in each sampling area.

Pathogens	Sampling areas			
	Messina ($n=23$)	Sassari ($n=111$)	Bari ($n=57$)	Total ($n=191$)
Gastrointestinal parasites				
Toxocara cati	5 (21.7%)	13 (11.7%)	11 (19.3%)	29 (15.2%)
Ancylostoma tubaeforme	14 (60.9%)	9 (8.1%)	0	23 (12%)
Cystoisospora felis	2 (8.7%)	21 (18%)	0	23 (12%)
Taenia taeniformis	0	4 (3.6%)	0	4 (2.1%)
Dipylidium caninum	2 (8.7%)	0	7 (12.3%)	9 (4.7%)
Giardia lamblia	0	9 (8.1%)	0	9 (4.7%)
Strongyloides spp.	0	1 (0.9%)	0	1 (0.5%)
Lungworms				
Aelurostrongylus abstrusus	11 (47.8%)	22 (19.8%)	1 (1.8%)	34 (17.8%)
Troglostrongylus brevior	1 (4.3%)	2 (1.8%)	3 (5.3%)	6 (3.1%)
Eucoleus aerophilus	1 (4.3%)	0	0	1 (0.5%)

3. Results

3.1. Prevalence of endoparasite infections in cats at the enrolment

Results of faecal examinations are reported in Tables 1 and 2. Out of 191 cats examined (i.e., 84 male, 107 female), 87 (45.5%) were positive for either gastrointestinal parasites and/or lungworm nematodes. In particular, 29 cats (15.2%) were infected by *T. cati*, 23 (12%) by *A. tubaeforme*, 23 (12%) by *Cystoisospora felis*, 9 (4.7%) by *G. lamblia*, 13 (6.8%) by tapeworms (i.e., 9 *D. caninum* and 4 *T. taeniformis*) and 1 (0.5%) by *Strongyloides* spp. In addition, 34 animals (17.8%) were infected by *A. abstrusus*, 6 (3.1%) by *T. brevior*, and one cat (0.5%) by *Capillaria aerophila* (syn. *Eucoleus aerophilus*). Mixed-infections by two or more pathogens were reported in 35 (18.3%) animals (Table 2) and one cat was co-infected by the three lungworm species (i.e., *A. abstrusus*, *T. brevior* and *E. aerophilus*), and two cats were infected by *A. abstrusus* and *T. brevior*.

3.2. Evaluation of treatment against lungworms

Twenty-three cats (13 male and 10 female), ranging from 2 months to 7 years of age, were positive for *A. abstrusus* ($n=18$) or *T. brevior* ($n=3$) or for both species ($n=2$) and were enrolled in the efficacy study (Fig. 1). In particular, 17 (73.9%) were co-infected with up to three gastrointestinal parasites: 7 animals (30.4%) were positive for *A. tubaeforme*; 2 (8.7%) for tapeworms (one *D. caninum* and one *T. taeniformis*); 1 (4.3%) for *T. cati*, 1 for *A. tubaeforme* and *D. caninum*; 2 for *A. tubaeforme* and *T. cati*, 1 for *T. taeniformis* and *T. cati*, 1 for *A. tubaeforme*, *T. cati* and *E. aerophilus*, 2 for *A. tubaeforme*, *T. cati* and *C. felis*.

Table 2
Frequency of diagnosis of single or mixed parasite infections in cats of different age groups.

Parasite species	<6 months ($n=77$)	6 months–2 years ($n=59$)	>2 years ($n=55$)	Total ($n=191$)
Single infections				
Toxocara cati (*T. c*)	6 (7.8%)	2 (3.4%)	0	8 (4.2%)
Dipylidium caninum (*D. c.*)	0	1 (1.7%)	2 (3.6%)	3 (1.6%)
Taenia taeniformis (*T. t*)	0	0	1 (1.8%)	1 (0.5%)
Ancylostoma tubaeforme (HW)	1 (1.3%)	1 (1.7%)	1 (1.8%)	3 (1.6%)
Cystoisospora felis (*C. f.*)	11 (14.3%)	0	1 (1.8%)	12 (6.3%)
Giardia lamblia (*G. l.*)	5 (6.5%)	2 (3.4%)	0	7 (3.6%)
Lungworms (LW)	5 (6.5%)	7 (9.1%)	4 (7.3%)	16 (8.4%)
Strongyloides spp.	0	0	1 (1.8%)	1 (0.5%)
Mixed infections				
T. c. + HW	0	1 (1.7%)		1 (0.5%)
T. c. + TW	1 (1.3%)	1 (1.7%)	2 (3.6%)	4 (2.1%)
T. c. + LW	1 (1.3%)	1 (1.7%)		2 (1.1%)
T. c. + *C. f.*	5 (6.5%)	0	0	5 (2.6%)
G. l. + LW	1 (1.3%)	0	0	1 (0.5%)
HW + LW	0	7 (9.1%)	1 (1.8%)	8 (4.2%)
T. t. + LW	0	0	1 (1.8%)	1 (0.5%)
D. c. + HW	0	0	1 (1.8%)	1 (0.5%)
T. c. + HW + *C. f.*	2 (2.6%)	0	0	2 (1.1%)
T. c. + *D. c.* + LW	3 (3.9%)	0	0	3 (1.6%)
T. c. + HW + LW	0	1 (1.7%)	0	1 (0.5%)
D. c. + HW + LW	0	1 (1.7%)	1 (1.8%)	2 (1.1%)
HW + *C. f.* + LW	0	0	1 (1.8%)	1 (0.5%)
T. c. + HW + *C. f.* + *G. l.*	1 (1.3%)	0	0	1 (0.5%)
T. c. + HW + *C. f.* + LW	1 (1.3%)	0	1 (1.8%)	2 (1.1%)

Fig. 1. First-stage larvae (L1) of *Troglostrongylus brevior* (A) and *Aelurostrongylus abstrusus* (B) (Scale bar = 50 μm).

Table 3
Lugworm larval counts before and 28 ± 2 days after treatment, percentage efficacy and results of data analysis.

Lungworms	Mean number of Lpg[a] (range)					
	Pre-treatment[b]		Day 28 (±2)		% Efficacy[c]	*p*-value[d]
	NI/N[e]	GM[f] (range)	NI/N[e]	GM[f] (range)		
A. abstrusus	20/23	43.9 (1–1700 Lpg)	8/23	4.2 (0–1000 Lpg)	90.5	<0.0001
T. brevior	5/23	23.9 (3–85 Lpg)	0/23	0	100	<0.0001

[a] Lpg = larvae per gram of faeces.
[b] Treatment = Broadline®.
[c] Percentage efficacy = 100[(*C* − *T*)/*C*], where *C* and *T* are the geometric mean faecal larvae counts prior to treatment and at Day 28 (±2), respectively.
[d] Probability value analysed by modelling the logarithm of the counts +1 by a repeated measures analysis of variance where the measurement time (pre-treatment versus post-treatment) was the repeated measurement within the individual animal.
[e] NI/N = Number of cats that had evidence of the indicated parasite per number of cats treated.
[f] GM = geometric mean.

Faecal nematode larval counts before and after treatment (day 28 ± 2), percentage efficacy and results of data analysis are reported in Table 3. At the enrolment, cats shed a mean of 43.9 or 23.9 Lpg of *A. abstrusus* or *T. brevior*, respectively. Seven cats (30.4%) infected by *A. abstrusus* displayed severe respiratory signs (i.e., dyspnoea, recurrent cough, nasal discharge, wheezing), while one animal co-infected by *T. brevior* and *T. cati* had diarrhoea.

Post-treatment larval counts of both metastrongyloid species were significantly reduced ($p < 0.0001$) and clinical respiratory signs resolved in all treated animals. Fifteen (65.2%) cats tested negative for lungworm larvae after a single treatment. The overall efficacy with Broadline® was 90.5% or 100% for *A. abstrusus* or *T. brevior*, respectively. In addition, 20 (87%) cats were negative for gastrointestinal parasites whereas three (13%) shed eggs of *A. tubaeforme*, *E. aerophilus* or *T. cati*, respectively. No health problems related to the treatment were observed.

4. Discussions

The high prevalence of the infection for any helminthic parasite (i.e., 45.5%) suggests that cats living in the regions examined are exposed to the infection with several pathogen species throughout their life, according to their age, life style and the environment where they live. Indeed, kittens tend to be more often infected by protozoans (e.g., *C. felis* and *G. lamblia*) and ascarids (i.e., *T. cati*), than adult cats. This trend has been confirmed in studies on *T. cati* and *C. felis*, in which it has been shown that infected cats ≤9 months of age are at higher risk for those pathogens (Beugnet et al., 2014; Knaus et al., 2014b). Conversely, in the present study the age of animals was not a risk factor for lungworm infection, since either young or adult animals were infected by *A. abstrusus* or *T. brevior*. However, the severity of troglostrongylosis may be correlated with the age of the individual, with fatal outcomes observed in kittens (Brianti et al., 2014; Giannelli et al., 2014a,b). Further studies should assess whether the occurrence of mixed infections increase the pathogenicity of feline metastrongyloids. However, there is an indication that simultaneous infections with more than one species of lungworms may worsen the severity of clinical signs (Varcasia et al., 2015).

Results of this study indicate that a single administration of the combination of fipronil, (*S*)-methoprene, eprinomectin and praziquantel is effective for the treatment of *A. abstrusus* (90.5%) and *T. brevior* (100%) infection

in cats living under field conditions. In addition, the same combination product was confirmed to be highly efficacious against naturally acquired gastrointestinal parasites (e.g., *T. cati*, hookworms, tapeworms), also in the case of multiple infections, under field conditions (Rehbein et al., 2014). In the present study, the enrolled cats probably harboured different developmental stages of lungworms, since they were infected under natural conditions. This complements the results obtained from a controlled laboratory study, in which the efficacy of the product against third- and fourth-stage larvae of *A. abstrusus* was assessed (Knaus et al., 2014a). Whether repeated monthly treatments would increase the efficacy of the product in cats infected by *A. abstrusus* should be defined.

In conclusion, Broadline® spot-on was proven to be highly effective against both *A. abstrusus* and *T. brevior* under field conditions, by reducing significantly larval shedding. All treated cats recovered from respiratory signs.

Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. Some authors (i.e., MK, LH, FB) are currently employed at Merial.

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Acknowledgment

The authors gratefully acknowledge the statistical support provided by S. Theodore Chester.

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