Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against adult and larval stages of Toxocara cati in cats

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ABSTRACT

The efficacy of 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) was evaluated against adult and larval Toxocara cati in four controlled studies. All studies included experimentally infected, purpose-bred, short-haired cats. In two studies, 22 or 20 cats harbouring patent infections as confirmed by pre-treatment faecal examination, were included. Within each study, cats were allocated to one of two groups: control or treated. In a further two studies, 30 cats were included in each; cats were allocated to one of three groups: control, treated when T. cati were expected to be either migrating third and/or fourth-stage larvae, or treated when T. cati were expected to be fourth-stage larvae. Cats allocated to the treated groups received a single topical application of the combination product at 0.12 ml/kg body-weight (10 mg fipronil + 12 mg (S)-methoprene + 0.5 mg eprinomectin + 10 mg praziquantel per kg). For parasite recovery and count, cats were euthanized humanely at different intervals after treatment. In the studies targeting adult T. cati, ascarids were recovered from all controls (range 1–150) while only two worms were isolated from one treated cat. Thus, the efficacy of the novel combination was 99.4% and 100% against adult T. cati. For studies targeting larval T. cati, up to 21 worms were recovered from each of seven or eight of the control cats per study. No T. cati were recovered from the treated cats in two studies, corresponding to 100% efficacy against both, migrating third and/or fourth-stage larvae and luminal fourth-stage larvae. All cats accepted the treatment well and no adverse experiences or other health problems were observed throughout the studies.

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1. Introduction

The ascarid Toxocara cati is a cosmopolitan parasite of felids and represents the most common gastrointestinal parasite of domestic cats worldwide (Bowman et al., 2002; Epe, 2009). Cats may become infected with T. cati by three different routes: by ingestion of larvated (infective) eggs, by ingestion of paratenic hosts infected with somatic larval T. cati, or by transmammary infection of suckling kittens. It appears likely that the predominant route of infection for the adult cat is the ingestion of paratenic hosts, such as rodents and birds while kittens may get infected soon after birth when ingesting colostrum or milk from their infected dam. The development of T. cati in the cat, as well as the
role of paratenic hosts, has been studied by several authors (reviewed by Anderson, 2000; Bowman et al., 2002).

While there are some reports on clinical signs in cats associated with ascarid infections like diarrhoea, potbelly, poor coat and failure to thrive, it is well accepted that the majority of cats do not show clinical signs (Aoki et al., 1990; Bowman et al., 2002). However a recent study demonstrated considerable pathology during pre-patent development (Dillon et al., 2013). In paratenic hosts as well as in other accidental hosts (Azizi et al., 2007; Davidson et al., 2012), the development of T. cati is limited basically to penetration and somatic migration of third-stage larvae and associated damage. Several reports support the hypothesis that along with other ascarids, T. cati must be considered as a zoonotic agent that may cause human toxocarosis exhibiting visceral larval migrans, ocular toxocarosis, neural larva migrans or covert toxocarosis (Fisher, 2003; Lee et al., 2010). In addition, epidemiological studies suggest associations of human toxocarosis to the development of allergic diseases, including asthma, or epilepsy (Pinelli et al., 2006; Pinelli and Aranzamendi, 2012; Quattrrochi et al., 2012). Infection risks for humans are the result of direct contact with infected cats or through the environment or food contaminated by the faeces of infected free-roaming cats (Overgauw et al., 2009; Klapeć and Borecka, 2012; Trejo et al., 2012). Periodic treatments with anthelmintics are considered an important measure to reduce the frequency of patent ascarid infections in cats and the risk of transmission of infection to both other pets and humans (Deplazes et al., 2011; Overgauw and van Knapen, 2013).

The four studies reported here were conducted to evaluate the efficacy of Broadline®, a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel, against adult and larval stages of T. cati in cats.

2. Materials and methods

The design of the studies was in accordance with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) – GL7, “Efficacy of Anthelmintics: General Requirements” (Vercruysse et al., 2001), VICH GL20 “Efficacy of Anthelmintics: Specific Recommendations for Felines” (Vercruysse et al., 2002), and the “World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats” (Jacobs et al., 1994). All studies were conducted in compliance with VICH GL9, entitled Good Clinical Practice and in compliance with local animal welfare legislation and were approved by an Independent Animal Care and Use Committee. All personnel involved in collecting efficacy data were blind to the treatment assignment of the animals.

2.1. Experimental animals and inoculation

2.1.1. Efficacy against adult T. cati

The studies against adult T. cati were conducted in the USA (Study 1) and Germany (Study 2) with 22 or 20 purpose-bred short-haired cats, respectively. Cats weighed between 1.23 and 2.91 kg prior to treatment and were approximately 12–21 weeks old, respectively (Table 1). The animals were either housed individually during the entire study (Study 1) or in groups until seven days prior to treatment and individually thereafter (Study 2). The environmental conditions were identical for all animals in a study. All cats were confirmed negative for ascarial eggs prior to inoculation by examination of faeces using standard coproscopic techniques.

In both studies, each cat was inoculated orally on three consecutive days (63, 62 and 61 days prior to treatment) with approximately 100 infective (larvated) Toxocara eggs per day. For inoculation, recent field isolates (as defined per VICH GL 7, Vercruysse et al., 2001) originating from the USA (Study 1) or Bulgaria (Study 2) were used (Table 1).

Faecal samples were collected either five or four days prior to treatment and examined to confirm the presence of Toxocara eggs using quantitative flotation techniques (Study 1: modified Wisconsin technique; Study 2: modified McMaster technique). All inoculated cats were shedding Toxocara eggs prior to treatment (Study 1: 226–4902 eggs per g of faeces [EPG]; Study 2: 1050–16,650 EPG) and were included in the studies.

2.1.2. Efficacy against larval T. cati

The studies against larval T. cati were conducted in Germany (Studies 3 and 4) and included 30 purpose-bred, short-haired cats each. Cats weighed between 1.0 kg and 2.5 kg prior to treatment and were approximately three to four months old, respectively (Table 1).

Cats were housed individually (Study 4) or in groups of six (Study 3) cats from study start until Day – 8. From Day – 7 onwards, all animals were housed individually. Cats were acclimated to the study facilities for seven days prior to treatment. The environmental conditions were identical for all animals in a study. All cats were confirmed to be negative for ascarial eggs by standard faecal examination techniques prior to inoculation.

For each study, cats were inoculated orally once with approximately 300 (Study 3), or 250 (Study 4) infective (larvated) Toxocara eggs each. For inoculation, recent field isolates (as defined per VICH GL 7, Vercruysse et al., 2001) originating from Bulgaria (Study 3), and Germany (Study 4) were used (Table 1). In addition, cats in Study 3 were inoculated with approximately 200 infective Ancylostoma tubaeforme larvae, each (results not shown here but presented by Prullage et al., 2014).

2.2. Study design

The studies utilized a randomized block design based on pre-treatment bodyweight. In the studies conducted to evaluate the efficacy against adult T. cati, cats were ranked based on decreasing Day – 3 bodyweight and placed in replicates of two cats, each. Within replicates, cats were
Table 1
Characteristics of experimental animals and study design including day(s) of inoculation, number of *Toxocara cati* eggs provided, day of treatment and day of necropsy.

<table>
<thead>
<tr>
<th>Inoculation of cats</th>
<th>Treatment group</th>
<th>Animal data</th>
<th>Treatment</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation days</td>
<td>Total number of larvated <em>T. cati</em> eggs inoculated</td>
<td>Age at treatment</td>
<td>Sex</td>
<td>Pre-treatment bodyweight (kg)</td>
</tr>
<tr>
<td>Study 1</td>
<td>Days −63, −62 and −61 ∼300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated Treated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12–14 weeks 10 Male, 1 female 1.89–2.81</td>
<td>–</td>
</tr>
<tr>
<td>Study 2</td>
<td>Days −63, −62 and −61 ∼300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated Treated</td>
<td>18–21 weeks 6 Male, 4 female 1.248–2.290</td>
<td>–</td>
</tr>
<tr>
<td>Study 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Day −5 &lt;∼300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated Treated</td>
<td>13–14 weeks 5 Male, 5 female 1.035–1.538</td>
<td>–</td>
</tr>
<tr>
<td>Study 4</td>
<td>Day −5 &lt;∼250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated Treated</td>
<td>3–4 months 5 Male, 5 female 1.290–2.080</td>
<td>Day 0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Broadline<sup>®</sup> spot on for cats = fipronil (8.3%, w/v), (S)-methoprene (10%, w/v), eprinomectin (0.4%, w/v), and praziquantel (8.3%, w/v); at 0.12 mL/kg body weight.

<sup>b</sup> Origin of isolate: naturally infected cat from Tennessee, USA; isolated eggs were incubated and directly used within two months from isolation.

<sup>c</sup> Origin of isolate: naturally infected cat from Bulgaria; isolate maintained for three years in laboratory through passages in cats.

<sup>d</sup> Cats additionally inoculated with ∼200 *A. tubaeforme* larvae on Day −7; data shown in Prullage et al. (2014).

<sup>e</sup> Origin of isolate: naturally infected cat from Germany; isolated eggs were incubated and directly used within six months from inoculation.

<sup>f</sup> *T. cati* expected to be migrating third and/or fourth-stage larvae.

<sup>g</sup> *T. cati* expected to be fourth-stage larvae.
allocated randomly to one of two groups: one to the untreated control group and one to the group treated with the novel topical formulation. In the studies conducted to evaluate the efficacy against larval *T. cati*, cats were ranked based on decreasing Day – 3 or – 1 bodyweight and placed in replicates of three cats, each. Within replicates, cats were allocated randomly to one of three treatment groups: untreated (control) group, a group treated when *T. cati* were expected to be migrating third and/or fourth-stage larvae (five days post inoculation), or a group treated when *T. cati* were expected to be luminal fourth-stage larvae (19 days post inoculation) (Vercrieuysse et al., 2002).

The treatment, fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v), praziquantel 8.3% (w/v), (Broadline®, Merial), was administered once topically at the minimum therapeutic dose of 0.12 mL/kg bodyweight 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 4 h post-treatment and thereafter once daily until the end of the study for health problems or adverse events.

### 2.3. Parasite recovery and count

For parasite recovery and count, cats were euthanized humanely at different intervals after treatment (Table 1). The content of the whole gastrointestinal tract (including stomach, small and large intestine) was collected. To facilitate the isolation and counting of ascarids, organ contents were washed through appropriate sized sieves to remove debris. Counts were made on total gastrointestinal contents. Parasites were identified to species and stage based on morphological characteristics.

### 2.4. Data analysis

For all studies, parasite counts (adult *T. cati*, Studies 1 and 2; total *T. cati*, Studies 3 and 4) 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 100[(C – T)/C], where C was the geometric mean of the parasite count for the untreated (control) animals and T was the geometric mean of the parasite count of the treated group. The log-counts of each treated group were compared to the log-counts of the untreated (control) group using a F-test adjusted for the allocation blocks used to randomize the animals to the treatment groups. The Mixed procedure in SAS® was used for the analysis with the treatment groups defined as a fixed effect and the allocation blocks defined as a random effect. All testing was two-sided at the $\alpha = 0.05$ significance level.

### 3. Results

No adverse experiences or other health problems were observed after treatment application or throughout the studies, indicating that the treatment was well accepted.

The results of the studies are summarized in Table 2. Between 1 and 150 adult *T. cati* were recovered from the untreated cats in studies 1 and 2, while only 2 *T. cati* were recovered from one of the cats treated with the novel combination in Study 1. The therapeutic efficacy of the novel topical combination product against adult *T. cati* was thus 99.4% in study 1 and 100% in study 2 ($P < 0.001$). In addition to adult worms, small numbers of immature *T. cati* were recovered from some cats in both studies (1–14 larvae per cat in 9 of 11 untreated animals vs. 1 larva in 1 of 11 treated animals in Study 1; 1–7 larvae each in 8 of 10 untreated animals vs. 3 larvae in 1 of 10 treated animals in Study 2). The mean establishment rate (percentage ratio of number of total nematodes in controls found at necropsy vs. number of eggs inoculated) was 13.2% and 27.6% in Studies 1 and 2, respectively, with 89.7% and 97.2% of the total *T. cati* burden consisting of adult parasites.

In Studies 3 and 4, seven and nine cats out of ten controls, respectively, harboured between 1 and 21 *T. cati* (fourth-stage larval and/or pre-adult stages), corresponding to establishment rates of 2.6% and 1.8%, respectively. Zero *T. cati* were recovered from the treated cats in Studies 3 and 4, corresponding to 100% efficacy ($P < 0.001$) against both migrating third and/or fourth-stage and luminal fourth-stage larvae.

All studies were considered valid as they met the recommendations as to adequacy-of-infection per VICH GL7 and

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**Table 2**

Parasite counts and therapeutic efficacy of Broadline® spot-on for cats against different developmental stages of experimentally induced *Toxocara cati*.

<table>
<thead>
<tr>
<th>Target parasite stage</th>
<th>Study</th>
<th>Untreated (control)</th>
<th>Topical FMEP $^a$</th>
<th>Efficacy (%) $^b$</th>
<th>P-value $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NI/NG $^d$</td>
<td>GM (range) $^e$</td>
<td>NI/NG</td>
<td>GM (range) $^e$</td>
</tr>
<tr>
<td>Adult <em>T. cati</em></td>
<td>1</td>
<td>11/11</td>
<td>17.43 (1–131)</td>
<td>1/11</td>
<td>0.11 (0–2)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10/10</td>
<td>70.9 (37–150)</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>Luminal fourth-stage T. cati larvae</td>
<td>3</td>
<td>7/10</td>
<td>5.14 (0–21)</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9/10</td>
<td>3.3 (0–10)</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>Migrating third and/or fourth-stage <em>T. cati</em> larvae</td>
<td>3</td>
<td>7/10</td>
<td>5.14 (0–21)</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9/10</td>
<td>3.3 (0–10)</td>
<td>0/10</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Topical fipronil (8.3%, w/v), (S)-methoprene (10%, w/v), eprinomectin (0.4%, w/v), and praziquantel (8.3%, w/v); at 0.12 mL/kg body weight.

$^b$ Efficacy = 100 [(geometric mean treated (control) group – geometric mean FMEP group)/geometric mean untreated (control) group].

$^c$ Two-sided p-value comparing the worm burden of the topical FMEP group with the control.

$^d$ NI/NG: Number of cats infected/number of cats in group.

$^e$ GM: geometric mean count (based on transformation to ln [count + 1]).

$^f$ Studies 1 and 2: adult *T. cati*; Studies 3 and 4: total *T. cati*. 

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VICH GL20 with at least six control animals harbouring at least five *T. cati* each.

### 4. Discussion

These studies confirmed the high therapeutic efficacy of topical fipronil, (S)-methoprene, eprinomectin and praziquantel against adult and larval *T. cati* infections. The results of these studies demonstrated that the combination product is efficacious against *T. cati* during the immature stages while the parasite is developing within the cat host. This feature is important as this effect contributes to a more sustainable prevention of ascariid infections in cats and thus reduces the risk of transmission of infection.

The high level of efficacy against both larval stages and adults *T. cati* in experimentally infected cats is supported by the results of other controlled studies using the novel topical combination formulation in naturally infected animals (Knaus et al., 2014) and under field conditions involving cats from seven countries in Europe (Rehbein et al., 2014).

Geometric mean total adult *T. cati* counts of the control animals in the four studies ranged from 3.3 to 70.9. These numbers are consistent with observations in other studies utilizing similar doses of 200–400 infective eggs for inoculation of cats (e.g., Reinemeyer et al., 2005; Schenker et al., 2007; Wolken et al., 2012). As indicated by the rates of establishment (including ratios of number of cats infected/number of cats in group) inoculation of higher doses of infective eggs as a single bolus (Studies 3 and 4) resulted in lower worm counts and a higher number of uninfectected cats compared to the inoculation of lower doses of infective eggs over three consecutive days (Studies 1 and 2). It has been known for quite some time that immunological reactions play a significant role in the host–parasite interaction during the initial phase of infection of cats with *T. cati* (Sarles and Stoll, 1935). Therefore, the findings from our studies suggest that single exposure to higher doses of infective *T. cati* eggs stimulates host reactions to a greater extent than does multiple exposures to smaller number of infective eggs that result in lower numbers of parasites undergoing development.

Macrocytic lactones have been used as anthelmintics in cats for several years and provide excellent efficacy against infections with various nematode parasites of pets (reviewed by Guerrero et al., 2002; Nolan and Lok, 2012). The results suggest that the novel topical combination product containing eprinomectin will provide an efficacious, safe and convenient solution for the treatment of adult and larval stages of *T. cati* in cats.

### Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. All authors are current employees or contractors of Merial.

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### References


