Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against experimental infections of *Toxascaris leonina* in cats

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\textbf{A R T I C L E  I N F O}

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Praziquantel

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Nematodes

Cat

\textbf{A B S T R A C T}

The efficacy of a novel topical fipronil, (S)-methoprene, eprinomectin and praziquantel combination product (BROADLINE\textsuperscript{®}, Merial) was evaluated against adult *Toxascaris leonina* ascarids in experimentally infested cats in two controlled studies under an identical protocol. For each study, 30 nematode-naive, purpose-bred European Short Hair cats were inoculated orally with approximately 300 larvated *T. leonina* eggs. Twenty-two and 24 cats, respectively, that were shown to be positive for *Toxascaris* eggs by pre-treatment faecal examination were subsequently included in the two studies. In each study, the animals were allocated randomly to an untreated (control) group or to a treatment group. The treatment was a novel topical combination: fipronil (8.3\%, w/v), (S)-methoprene (10\%, w/v), eprinomectin (0.4\% w/v) and praziquantel (8.3\% w/v). Treatment was applied on Day 0 at 0.12 mL/kg bodyweight. For parasite recovery and count, cats were euthanized humanely seven days after treatment and necropsied. All untreated cats harboured adult *T. leonina* (range, 1–31 nematodes). The treatment provided a high level of efficacy against adult *T. leonina* in both studies (95.8\% and 98.1\%, respectively p < 0.001). All cats accepted the treatment well based on hourly post-treatment observations for 4h and daily observations thereafter. No adverse experiences or other health problems were observed throughout the studies. Thus the data indicate that this novel combination product will provide a safe and effective treatment against *T. leonina* in cats.

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1. Introduction

There are two species of ascarids affecting cats and kittens: *Toxocara cati* and *Toxascaris leonina*. *Toxocara cati* is the most common nematode parasite of cats while *T. leonina* is less common. In contrast to *Toxocara cati* being specific to cats, *T. leonina* can complete its life cycle in several host species, primarily including domestic and wild canids (dogs and foxes), but it also infects felids (Sprent, 1959; Bowman \textit{et al}., 2002).

*T. leonina* infections have been demonstrated in several cat parasite surveys worldwide (Parsons, 1987). Relevant information on the current occurrence of *Toxascaris* infections in domestic cats of different categories is available through coproscopical and autopsy surveys conducted on all continents: Africa (Khalafalla, 2011; Sowemimo, 2012), Asia (e.g. Schuster \textit{et al}., 2009; Abu-Madi \textit{et al}., 2010; Borji \textit{et al}., 2011; Al-Obaidi, 2012; Itoh \textit{et al}., 2012), Australia

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The healthy, against treatment of indicating “Efficacy 2009; 2011; Mugnaini et al., 2012; Năreaho et al., 2012), North America (Gates and Nolan, 2009; Blagburn et al., 2012) and South America (Labarthe et al., 2004; Sommerfelt et al., 2006). Rates of T. leonina infection show large variability between surveys with generally lower frequencies observed in the northern hemisphere. The highest prevalence reported was 30% through autopsy of cats (Al-Obaidi, 2012) or 23.5% by faecal examination (Sowemimo, 2012). As with Toxocara cati, younger cats are more likely to harbour T. leonina infections (McGlade et al., 2003; Capári et al., 2013). Concomitant Toxascaris and Toxocara infections appear to be very rare in cats (Parsons, 1987). However, an exceptionally high rate of concurrent Toxascaris and Toxocara presence has been described in the cat population of a farm in England (Yamaguchi et al., 1996), indicating special endemic and close contact conditions. Although infections of cats by T. leonina may be well tolerated and there is no evidence of zoonotic potential for this parasite, infections should be controlled appropriately for hygienic reasons and to reduce the transmission of this parasite. Cat isolates are infectious for both cats and dogs while isolates derived from dogs have been shown to be unlikely to establish in cats (Sprent, 1959; Okoshi and Usui, 1968; Fukase et al., 1987).

The two studies reported here were designed to evaluate the efficacy of a novel topical combination formulation of fipronil, (S)-methoprene, eprinomectin and praziquantel against adult T. leonina ascarids in cats.

2. Materials and methods

The study design was in accordance with the International Co-operation 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 blinded to the treatment assigned to the animals.

2.1. Experimental animals

In each study, following an acclimation period, 30 healthy, purpose-bred European Short Hair cats were inoculated orally with Toxascaris eggs. At the start of the study, the cats were nematode-free as confirmed by standard faecal examination techniques prior to initial inoculation. Based on pre-treatment faecal examination, 22 and 24 cats were included in Study 1 and Study 2, respectively. The animals weighed between 1.214 kg and 3.178 kg prior to treatment and were approximately 5–8 months old (details are shown in Table 1). In both studies, cats were housed in group runs until nine days before treatment when they were transferred to individual cages until the end of the study. The environmental conditions were identical for all animals within a study.

2.2. Experimental infection and pre-treatment faecal examination

In each study, 30 cats were each inoculated orally on three consecutive days (72, 71 and 70 days prior to treatment) with approximately 100 infective (larvated) Toxascaris eggs per day using a disposable syringe. The Toxascaris eggs were extracted from the faeces of naturally infected cheetahs from a zoo located in Austria (Study 1) or a domestic cat living in a cattery in Hungary (Study 2). The eggs were incubated to become larvated and used for inoculation within 4 months from isolation.

Faecal samples were collected prior to treatment (Day 0) starting on Day −8 and examined to confirm the presence of Toxascaris eggs by using a modified McMaster technique (Wetzel, 1951) adjusted to a sensitivity of 25 eggs per gram of faeces (EPG). In Study 1, 22 of the 30 inoculated cats were demonstrated shedding Toxascaris eggs (200–1600 EPG) over a four-day period (nine, eight, four and one cat tested positive sequentially eight, seven, six or five days prior to treatment) and were included in the study. In Study 2, 28 of the 30 inoculated cats shed Toxascaris eggs (25–1950 EPG) when examined eight days prior to treatment, and the 24 cats with the highest faecal egg counts were included in the study. The cats not included in the study received anthelmintic treatment.

2.3. Experimental design

The studies were conducted under a single protocol utilizing a randomized block design based on pre-treatment body weight. Replicates of two cats were formed sequentially, based on decreasing pre-treatment (Day −2/−1) body weights. Within replicates, animals were randomly allocated to groups: one to the untreated (control) group and one to the treated group.

The treatment, fipronil (8.3%, w/v), (S)-methoprene (10%, w/v), eprinomectin (0.4%, w/v) and praziquantel (8.3%, w/v) (Broadline®, Merial) was administered topically at 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 once on Day 0. This dosage corresponded to the minimum label dose and delivered 10 mg fipronil + 12 mg (S)-methoprene + 0.5 mg eprinomectin and 10 mg praziquantel per kg of bodyweight.

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. For parasite recovery and count, cats were humanely euthanized seven days after treatment by applying a procedure that was in accordance with standard veterinary routine, and necropsied.
Table 1

<table>
<thead>
<tr>
<th>Study/group</th>
<th>Sexa</th>
<th>~Age (months)</th>
<th>Pre-treatmentb body weight (kg)</th>
<th>Pre-treatmentc Toxascaris faecal egg counts, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>7 M, 4 F</td>
<td>5–8</td>
<td>1.8–3.2</td>
<td>200–1200 EPGd</td>
</tr>
<tr>
<td>Topical FMEPc</td>
<td>6 M, 5 F</td>
<td>5–8</td>
<td>1.9–2.4</td>
<td>150–1600 EPG</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>6 M, 6 F</td>
<td>5–6</td>
<td>1.5–2.3</td>
<td>100–1950 EPG</td>
</tr>
<tr>
<td>Topical treatment</td>
<td>7 M, 5 F</td>
<td>5–6</td>
<td>1.2–2.2</td>
<td>100–1850 EPG</td>
</tr>
</tbody>
</table>

a M = male; F = female.
b Day – 1, Study 1 or Day – 2, Study 2 prior to treatment (= Day 0).
c Established on Days –8, –7, –6 or –5 (Study 1) or Day –8 (Study 2) prior to treatment (= Day 0).
d EPG = eggs per gram of faeces.
e 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.

2.4. Parasite recovery and count

The contents of the total gastrointestinal tract (stomach, small and large intestines including cecum) were collected. To facilitate the isolation and counting of nematodes, organ contents were screened over 300 μm mesh size sieves to remove the debris. Counts of parasites were made on total gastrointestinal contents. Nematodes were identified by species and stage of development using morphological characteristics described in Hartwich (1975).

2.5. Data analysis

Adult T. leonina counts were transformed to the natural logarithm of (count + 1) for calculation of geometric means for each treatment group. Efficacy for the treated group was calculated as the percent efficacy using the formula $100 \times (C - T)/C$, where $C$ is the geometric mean among untreated (control) animals and $T$ is the geometric mean among the treated animals treated. The log-counts of the treated group were compared to the log-counts of the untreated (control) group using an $F$-test adjusted for the allocation blocks. The Mixed procedure in SAS® version 9.1.3 was used for this analysis with Group as the fixed effect and allocation blocks as the random effect. Testing was two-sided at the $\alpha = 0.05$ significance level.

3. Results

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

Both studies met the recommendations as to adequacy-of-infection per VICH GL7 and VICH GL20, as ten of 11 or nine of 12 cats in the untreated (control) groups of Study 1 or 2, respectively, harboured more than the minimum number of five adult T. leonina per cat accepted as an adequate infection.

The results of the two studies are summarized in Table 2. Cats treated with the novel topical combination had significantly ($P < 0.001$) fewer adult T. leonina than the untreated controls with an overall >95% reduction of worm burden. In addition to adult worms, small numbers of fourth-stage T. leonina larvae were recovered from some cats in both studies (1–2 larvae per cat in 4/11 untreated animals vs. zero larvae in 11 treated animals in Study 1; 1 larva each in 5/12 untreated animals vs. 2 larvae in 1/12 treated animals in Study 2).

4. Discussion

All untreated control animals harboured adult T. leonina with similar establishment rates (percentage ratio of number of adult nematodes found at necropsy/number of larvated eggs inoculated – based on untreated (control) animals’ counts) of the two T. leonina isolates: 4.76% for Study 1 and 4.03% for Study 2. This indicated similar susceptibility of the cats to the different T. leonina isolates which derived either from a large felid (cheetah, Study 1) or domestic cats (Study 2). This finding is in line with the general conclusions drawn by Okoshi and Usui (1968) from their experiments in cats with eggs cultured from naturally infected large captive felids or domestic cats. In contrast to our findings, Fukase et al. (1987) found tenfold lower

Table 2

<table>
<thead>
<tr>
<th>Adult Toxascaris leonina counts</th>
<th>Efficacy (%)d</th>
<th>p-valuee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI/NG</td>
<td>GM (range)</td>
<td></td>
</tr>
<tr>
<td>Study 1</td>
<td>11/11</td>
<td>12.52 (4–25)</td>
</tr>
<tr>
<td>Study 2</td>
<td>12/12</td>
<td>8.33 (1–31)</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI/NG</td>
<td>GM (range)</td>
<td></td>
</tr>
<tr>
<td>Study 1</td>
<td>4/11</td>
<td>0.53 (0–5)</td>
</tr>
<tr>
<td>Study 2</td>
<td>2/12</td>
<td>0.16 (0–2)</td>
</tr>
</tbody>
</table>

a Broadline® = 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 untreated (control) group – geometric mean Topical FMEP group)/geometric mean untreated (control) group].
c Two-sided $p$-value comparing the worm burden of the Topical FMEP group with untreated (control) group.
d NI/NG: Number of cats infected/Number of cats in Group.
e Geometric mean count (based on transformation to $\ln [\text{count} + 1]$).
establishment rates when inoculating of cats with 5000 eggs of cat-derived T. leonina isolates in Japan. However, experimental infection of cats with isolates derived from large felids may be challenging and result in variable, and even erratic, establishment rates of T. leonina (Reinemeyer et al., 2005). The duration of the prepatent period also has been related to the claimed existence of isolates adapted to specific hosts or groups of hosts. Using cat-derived eggs for inoculation of cats, Sprent (1959) found a prepatent period of 74 days, Okoshi and Usui (1968) reported a prepatent period of 62–63 days, and Fukase et al. (1987) recorded patent infections in cats as early as 50 days following inoculation. Our studies were not designed to assess the prepatent period of T. leonina in cats; however, the results of the pre-treatment faecal examinations indicate a prepatent period of less than nine weeks for the isolates derived from both, cheetah and domestic cat, with some more variability in the former. Despite not being well defined, these findings may be useful for experimental infection study protocols, because induced ascarid infections, in general, are prone to spontaneous expulsion of the worms.

The results of these two studies using induced infections with T. leonina demonstrated a high level of efficacy of the novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel. These data complement the results of the studies which were conducted to evaluate the efficacy of Broadline® against Toxocara cati (Knaus et al., 2014a,b).

Conflict of interest

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

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