Efficacy of a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel against feline urinary bladder worm (Capillaria plica) infection

Martin Knaus\textsuperscript{a,}\textsuperscript{*}, Enstela Shukullari\textsuperscript{b}, Joseph Rosentel\textsuperscript{c}, Steffen Rehbein\textsuperscript{a}

\textsuperscript{a} Merial GmbH, Katharinenhof Research Center, 83101 Rohrdorf, Germany
\textsuperscript{b} Universitetti Bujqësor, Fakulteti i Mjeksisë Veterinare, Kodër Kamët, Tirana, Albania
\textsuperscript{c} Merial Limited, Duluth, GA 30096, USA

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

\textbf{Keywords:}
Capillaria plica
Eprinomectin
Praziquantel
Nematodes
Cat

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

Infection with urinary capillarid bladder worms has been observed in cats worldwide. Although considered as generally causing no or little harm, infection with urinary capillarids may be associated with clinical disease which requires an appropriate treatment including the use of anthelmintics. Therefore, the efficacy of a novel topical combination formulation of fipronil 8.3\% (w/v), (S)-methoprene 10\% (w/v), eprinomectin 0.4\% (w/v), and praziquantel 8.3\% (w/v) (BROADLINE\textsuperscript{\textregistered}, Merial) was evaluated against urinary capillarids in naturally infected cats. Sixteen European Short Hair cats (5 male, 11 female) with capillarid eggs in their urine pre-treatment were included in the study. At the time of treatment, the cats were approximately ten months to eight years old and weighed 1.6–3.6 kg. Cats were ranked based on decreasing bodyweight and then randomly allocated within replicates of two animals to one of the treatment groups. Each cat in the treated group received one topical application of the combination product at the minimum therapeutic dose of 0.12 ml/kg body weight delivering 10 mg fipronil + 12 mg (S)-methoprene + 0.5 mg eprinomectin + 10 mg praziquantel per kilogram of body weight while the cats allocated to the control group remained untreated. For parasite recovery, identification and count, cats were euthanized humanely 14 days after treatment. All untreated cats harboured Capillaria plica in their urinary bladders (range 4–12), while no capillarids were recovered from the eight treated cats. Thus, the efficacy of the novel topical combination against C. plica was 100\%. All cats accepted the treatment well based on post-treatment observations and daily observations thereafter. No adverse events or other health problems were observed during the study.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

1. Introduction

Urinary bladder Capillaria spp. infections of carnivores are reported to occur worldwide in wild canids as primary hosts but also in wild cats (Felis silvestris) and domestic cats (Felis catus). Information on the occurrence of urinary capillarids in domestic cats from Europe, the Americas, Australia and Japan is available through post-mortem surveys (e.g., Wagner, 1936; Zdun, 1937; Erlich, 1938; Zebrowska, 1961; Waddell, 1968; Haralampides, 1978; Butterworth and Burton, 1980; Thienpont et al., 1981; Wilson-Hanson and Prescott, 1982; Fujimori et al., 1983; Santa Cruz and Lombardero, 1987; Raschka et al., 1994; Schuster et al., 1997; Dieffenbacher, 2007; Krone

http://dx.doi.org/10.1016/j.vetpar.2014.02.038
0304-4017/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
et al., 2008) and only recently through reports of clinical cases (Finnerup, 1986; Linden et al., 1986; Bédard et al., 2002; Dantas et al., 2008; Whitehead, 2009; Pagnoncelli et al., 2011; Rossi et al., 2011). The literature refers to three urinary capillarids occurring in cats: Capillaria (syn. Pearsonema) feliscati, C. (P.) plica and C. travassoi. It is, however, not clear whether those parasites are distinct species or just phenotypes of one species (Bowman et al., 2002).

Apart from anecdotal reports of disease outbreaks in farmed foxes (e.g., Volkmar, 1930; Schmid, 1934; Petrov and Borovkova, 1942), urinary capillarids reportedly cause little pathology with no pathological findings related to capillarid infections of the lower urinary tract in the majority of infected dogs and cats, presumably related to a low worm burden. However, published case reports on cats suggest an association of urinary capillariosis with clinical signs including abdominal pain, fever, urinary incontinence, dysuria, straining and cystitis requiring an appropriate symptomatic treatment but also the use of anthelmintics as causative treatment (Finnerup, 1986; Linden et al., 1986; Dantas et al., 2008; Whitehead, 2009; Pagnoncelli et al., 2011; Rossi et al., 2011).

As the treatment of urinary capillarid infection in cats was based previously only on reports published on the use of various anthelmintics in individual cases, the study reported here was conducted to evaluate the therapeutical efficacy of a novel topical combination formulation of fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v), and praziquantel 8.3% (w/v) (Broadline®, Merial) against urinary capillarids in cats (Fig. 1).

2. Materials and methods

The study design 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). The study was conducted in compliance to VICH GL9, entitled Good Clinical Practice and in compliance with local animal welfare legislation and was approved by an Independent Animal Care and Use Committee. All personnel involved in collecting efficacy and safety data were blinded to the treatment assigned to the animals.

2.1. Experimental animals

The study was conducted in Albania with 16 short haired cats. The cats weighed 1.6–3.6 kg prior to treatment (Day –3) and were approximately ten months to eight years old (Table 1). The animals were individually housed during the entire study and acclimated to the study facilities for seven days prior to treatment. The environmental conditions were identical for all animals in the study.

2.2. Selection of animals based on pre-treatment urine examination

The selection criterion for inclusion of cats in the study was the presence of a patent infection with urinary capillarids as confirmed by the demonstration of Capillaria eggs in urine samples collected during the acclimation period. Urine samples were collected from sedated cats five days prior to treatment by gentle bladder expression, and the urine sediment was examined microscopically for Capillaria eggs.

2.3. Experimental design

The study utilized a randomized block design based on pre-treatment body weight. Replicates of two cats each were formed sequentially based on decreasing pre-treatment body weights. Within replicates, cats were randomly allocated to treatments: one to the untreated (control) group and one to the group treated with Broadline®. The treatment was applied at the minimum therapeutic dose of 0.12 ml/kg body weight (fipronil [10 mg/kg], (S)-methoprene [12 mg/kg], eprinomectin [0.5 mg/kg], praziquantel [10 mg/kg]) 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. All cats were observed hourly for four hours post-treatment and thereafter once daily until end of the study for health problems or adverse events. Fourteen days after treatment, all cats were humanely euthanized and necropsied, and the nematodes in the urinary bladder were counted.

2.4. Parasite recovery and count

Nematode recovery counts were made on total urinary bladder contents, including parasites recovered from the epithelium of the bladder. The content of each urinary bladder

---

1 Broadline® is a trademark of Merial; all other marks are the property of their respective owners.
was examined under a dissecting microscope and nematodes recovered. Thereafter, the urinary bladders were soaked overnight in saline at approximately 38 °C. The next morning, the soak as well as the rinse of the bladder epithelium were examined as described above. As a last step, the epithelium of each bladder was carefully peeled off and washed through a 25 μm mesh sieve for the collection of remaining nematode parasites. Parasites were identified to species based on their morphology (Skrijabin et al., 1970).

2.5. Data analysis

Parasite 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 determined by calculating the percent efficacy as 100[(C–T)/C], where C was the geometric mean among untreated (control) cats and T was the geometric mean among the treated animals. 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 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 significance level α = 0.05.

3. Results

No capillarids were recovered from the eight treated cats, while C. plica was recovered from the urinary bladders of all untreated (control) cats. The efficacy of the novel topical combination product against C. plica was therefore 100% (P < 0.001) (Table 1). The study was considered valid as it met recommendations as to adequacy-of-infection per VICH GL7 and VICH GL20 (Vercruysse et al., 2001, 2002) with all eight cats in the control group harbouring four to twelve C. plica. No adverse experiences or other health problems were observed after treatment application and throughout the study, indicating that the treatment was well accepted.

4. Discussion

Despite documentation in cat populations worldwide, urinary capillarid infections do not appear to be common in domestic cats. However, due to the localization of the parasites in the urinary bladder and the passage of the eggs in the urine, urinary capillarid infection may not be diagnosed by routine parasitological examination; their collection from urine is challenging, and it can be assumed that the presence of bladder worms is likely to be easily overlooked.

Usually, the urinary capillarid burden is low in cats. In the present study a maximum of 12 worms were recovered which is in line with counts reported by other authors who found up to 25 urinary capillarids in domestic cats in Europe (Zdun, 1937; Erlich, 1938, Raschka et al., 1994; Schuster et al., 1997; Krone et al., 2008), Argentina (Santa Cruz and Lombardero, 1987) and Australia (Waddell, 1968; Wilson-Hanson and Prescott, 1982). Other studies by the present authors conducted in Albania, revealed C. plica in the urinary bladder of 38 of 57 cats examined with individual counts up to 71 worms. However, almost 90% of the infected cats harboured no more than 25 worms (Knaus et al., unpublished data).

For C. plica, which is mainly a parasite of wild canids like foxes, local prevalence can be as high as 98% (Bork-Mimm and Rinder, 2011). Although not completely accepted, annelids (earthworms) are considered as obligate intermediate hosts and are necessary for the first-stage C. plica larvae to become infectious to definitive hosts. Capillaria plica derived from foxes and passed through earthworms have been experimentally proven to be infective for cats (Petrov and Borovkova, 1942; Enigk, 1950). As earthworms are only very rarely eaten by cats, it has been suggested that common prey species of cats that do feed on earthworms may play an important role as paratenic hosts in the epidemiology of feline capillariosis (Prescott, 1984; Capári et al., 2013).

To date, there is no product with a registered indication against urinary capillarids in cats and there appears to be no reports on anthelmintics which were evaluated for this indication. Successful treatment of urinary capillariosis of individual dogs and cats using macrocyclic lactone compounds off-label by subcutaneous injection has been reported previously (Linden et al., 1986; Kirkpatrick and Nelson, 1987; Whitehead, 2009; Pagnoncelli et al., 2011). The results reported here indicate that the novel topical combination formulation is the first product containing a macrocyclic lactone (eprinomectin) whose efficacy in this indication was demonstrated in a well-controlled study. The results of the present study are complemented by the findings reported by Rehein et al. (2014) who report

---

Table 1
Characteristics of experimental animals, Capillaria plica counts and therapeutic efficacy in cats of Broadline® spot on against naturally acquired urinary bladder capillarids.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>~Age (months)</th>
<th>Pre-treatment body weight (kg)</th>
<th>Capillaria plica counts</th>
<th>Efficacyf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (control)</td>
<td>2 M, 6 F</td>
<td>24–84</td>
<td>1.6–3.6</td>
<td>8/8</td>
<td>100% (p &lt; 0.001)</td>
</tr>
<tr>
<td>Treated³</td>
<td>3 M, 5 F</td>
<td>10–96</td>
<td>1.8–3.5</td>
<td>0/8</td>
<td>0</td>
</tr>
</tbody>
</table>

a M = male, F = female.

b N/NG: Number of cats infected/number of cats in group.

c Geometric mean count (based on transformation to ln [count + 1]).

d Efficacy = 100 [geometric mean untreated (control) – geometric mean topical FMEP]/geometric mean untreated (control)].

e Two-sided p-value comparing the worm burden of the treated group with untreated control.

² 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.
excellent efficacy of the novel combination product against feline capillarids based on faecal egg count reduction in a field study setting involving cats from seven countries in Europe. However, capillarid eggs diagnosed in the faeces of cats in that study were unlikely to be derived from bladder worms but most probably originated from capillarids residing in the lungs.

Conflict of interest

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

Acknowledgments

The authors gratefully acknowledge the technical assistance of R. Winter and thank Dr. S.T. Chester for statistical analysis of the data. The authors gratefully acknowledge Lenaïg Halos and Frédéric Beugnet for the editorial review of the manuscript.

References