A pilot trial evaluating the efficacy of a 10% imidacloprid/2.5% moxidectin spot-on formulation in the treatment of natural nasal capillariosis in dogs

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A B S T R A C T

The efficacy and safety of a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) were evaluated in a pilot trial for the treatment of canine nasal capillariosis caused by Capillaria boehmi (syn. Eucoleus boehmi). Sixteen dogs copromicroscopically positive for C. boehmi eggs were confirmed, either by rhinostomy or species-specific PCR-coupled sequencing assays, as being affected by nasal capillariosis. The animals were randomly allocated to two different study groups, i.e. one (Group T) treated with Advocate® and one (Group C) left untreated, in a ratio of 1:1. The animals underwent clinical examination and quantitative copromicroscopy for C. boehmi eggs on Days -6 and -2 (baseline) and Day 28 ± 2 (post-baseline). Animals in Group T received Advocate® on Day 0. On Day 28 ± 2 the efficacy of the treatment (Group T) or the persistence of the infection (Group C) was confirmed by rhinostomy or, alternatively, by molecular procedures. Seven of the eight dogs in Group T were negative on Day 28 ± 2 (reduction of baseline faecal egg counts by 99.14%), while for one dog a second treatment on Day 28 ± 2 was necessary to clear the infection, as demonstrated on Day 56 ± 2 (reduction of baseline faecal egg counts by 100% in Group T). Seven animals in Group C received a rescue dose of Advocate® on Day 28 ± 2 and scored microscopically and molecularly negative for the parasite on Day 56 ± 2, thus increasing the reduction of post-baseline egg counts to 99.57% after a single administration. These promising results show that Advocate® spot-on is an effective formulation for the treatment of canine nasal capillariosis under field conditions.

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1. Introduction

Capillaria boehmi (syn. Eucoleus boehmi) is a capillarid nematode inhabiting the nasal turbinates and the frontal and paranasal sinuses of wild (e.g. foxes and wolves) and domestic canids. Since the 1980s, when cases of “nasal capillariosis” due to the closely related nematode Capillaria aerophila (syn. Eucoleus aerophilus) were published (Evinger et al., 1985; King et al., 1990), this parasite has been repeatedly described in the temperate regions of
North America and Europe (Campbell and Little, 1991; Schoning et al., 1993; Sřeret et al., 2003; Gajewska et al., 2004; Baan et al., 2011). Nonetheless, knowledge of the features of the disease, i.e. biological cycle, routes of transmission, epidemiology, clinical impact, remains scanty (Campbell and Little, 1991; Conboy, 2009). It is thought that animals acquire the infection by ingesting larvated eggs from the environment, and then larval stages migrate to the nasal cavities where they reach adulthood (Conboy, 2009). It has also been hypothesized, although never demonstrated, that larval C. boehmi might develop in earthworms acting as facultative intermediate or paratenic hosts (Campbell and Little, 1991), as has also been speculated for C. aerophila (Conboy, 2009; Traversa et al., 2010).

The infection caused by C. boehmi in dogs is either subclinical or clinically manifest when the damage in the epithelium of the nasal turbinates and sinuses induces rhinitis characterized by symptoms of varying severity, i.e. sneezing, reverse sneezing, nasal discharge and impairment of scenting ability (i.e. hypo- or anosmia) (Evinger et al., 1985; Campbell and Little, 1991; Piperisova et al., 2010; Baan et al., 2011; Veronesi et al., 2013). Furthermore, C. boehmi has recently been recognised as a potential cause of intracranial disease and meningoencephalitis in dogs as a result of aberrant migration in the cranial cavity (Clark et al., 2013).

Although C. boehmi is rarely detected in dogs, recent reports have suggested the spread of symptomatic infections in both the Americas and Europe (Piperisova et al., 2010; Baan et al., 2011; Di Cesare et al., 2012a; Magi et al., 2012; Clark et al., 2013; Veronesi et al., 2013). It is thus possible that C. boehmi is another non-intestinal nematode of dogs which is potentially emerging in several areas, as recently indicated for other respiratory parasites affecting dogs and/or cats (Traversa et al., 2010).

There is significant merit in evaluating effective therapeuti- c options for this neglected disease, in that no drug has been approved for the treatment of C. boehmi infection. The little information available is related to a few single clinical cases or small case series, most of which have evaluated macrocyclic lactones (MLs) with promising results (Evinger et al., 1985; Conboy, 2009; Veronesi et al., 2013; Conboy et al., 2013). In particular, moxidectin was recently shown to be effective in a single dog infected by C. boehmi (Veronesi et al., 2013) and in cats infected with the closely related C. aerophila (Traversa et al., 2012).

The pilot trial described here evaluated the efficacy and safety of a spot-on formulation containing 10% imidacloprid/2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) in the field treatment of canine nasal capillariosis.

2. Materials and methods

2.1. Pre-inclusion screening

The study was carried out from November, 2012 to June, 2013 in Italy following pre-inclusion screening of 287 dogs. The majority of the animals were kept in public or private kennels located in Central Italy and in particular in the municipalities of Latina and Rome (Latium region), Perugia (Umbria Region), Cesena (Emilia Romagna region) and Chiusi (Tuscany region), selected on the basis of previous history of suspected or diagnosed cases of nasal capillariosis. The others were dogs referred to veterinary hospitals for disorders of the upper respiratory tract or privately owned dogs whose faeces were examined by routine copromicroscopic at the Parasitological Unit of the Faculty of Veterinary Medicine in Perugia.

A faecal sample from each dog was collected and examined for C. boehmi eggs using a qualitative copromicroscopic concentration-flotation procedure with a sugar solution with 1.200 specific gravity (s.g.) (Sloss et al., 1994). The eggs of C. boehmi were identified on the basis of the following morphological and morphometric features: size 55.30 ± 1.30 × 32.40 ± 2.60 μm, a typical space between the embryo and the wall, asymmetry of the non-ringed plugs and the appearance of the egg shell characterized by several tiny pits (Di Cesare et al., 2012a).

All dogs which scored positive for eggs of C. boehmi (Fig. 1) at this qualitative copromicroscopic screening were submitted to confirmatory rhinoscopy to dem- onstrate the presence of the parasite in situ and/or to nasal flushing. If the owners did not consent the rhinoscopic procedure, a confirmatory species-specific PCR-coupled sequencing assay was used on the faecal samples. Briefly, the genomic DNA was extracted from each faecal sample and then subjected to a PCR assay specific for the mito- chondrial cox1 gene Capillariinae Subfamily as described previously (Di Cesare et al., 2012b). Additionally, DNA extracted from three adult specimens of C. boehmi micro- scopically identified at the species level was subjected

Fig. 1. Copromicroscopic examination: eggs of Capillaria boehmi showing the typical asymmetry of bipolar plugs and a small, clear to golden space between the embryo and the egg shell. Original magnification 40 X. Scale bar: 20 μm.
to the aforementioned PCR (Di Cesare et al., 2012b). The amplicons from both the adults and faecal eggs were sequenced and the sequences were compared with each other.

Dogs treated within the last two months with any anthelmintic drug, affected by severe systemic diseases or in generally poor health were excluded from the trial.

2.2. Animal enrolment, clinical examinations and anthelmintic treatments

Of the 287 dogs, 19 scored positive in copromicroscopy and rhinoscopy/confirmatory PCR, and 16 were enrolled in the study with the owner’s consent. The enrolled dogs consisted of nine privately owned animals and seven kennelled dogs of variable breed and gender with an age ranging from 1.5 to 10 years and weighing between 13.5 and 45 kg.

The dogs underwent two quantitative faecal egg counts (FECs) using a McMaster technique (Sloss et al., 1994) on Days -6 and -2 to ensure that a pre-existing C. boehmi infection was still present at the time of treatment. At the same time as the faecal samples were obtained, a pre-treatment clinical examination was conducted to detect symptoms compatible with nasal capillariasis. An individual form was completed for each dog to record its medical history and clinical data.

The 16 dogs were allocated to two different study groups, i.e. Group T treated with Advocate® and the control Group C left untreated, according to a randomized block design in a ratio of 1:1.

Dogs in Group T were treated topically on Day 0 with a single dose of Advocate®, with a second treatment planned for those which were still positive for C. boehmi eggs on Day 28 ± 2. All treated animals were clinically examined for 2–4 h post-treatment to evaluate the safety and the potential side effects of the spot-on formulation administered. The occurrence of adverse effects (AE) and serious adverse effects (SAE) was to be registered.

Dogs in Group C which were still infected on Day 28 ± 2 received a rescue dose of Advocate® and were examined for the persistence of the infection on Day 56 ± 2 as described above. Another clinical examination was conducted for all examined dogs on Days 28 ± 2 and 56 ± 2.

2.3. Post-treatment evaluation

On Day 28 ± 2 two faecal samples were collected from the 16 dogs and underwent post-treatment examination using the McMaster technique. Additionally, where permitted by the owners, another rhinoscopy was performed to confirm the efficacy of the treatment (T Group) and the persistence of the parasite (C Group). Alternatively, the above-mentioned molecular procedures were applied to faecal samples collected for post-treatment.

Dogs in both the groups which tested positive on Day 28 ± 2 underwent two further copromicroscopic examinations on Day 56 ± 2.

2.4. Data analysis

The primary efficacy criterion was the reduction of baseline eggs per gram (EPG) counts on Day 28 ± 2. The mean value from the two faecal counts performed in the pre-treatment assessment (i.e. two examinations on Day -6 and -2) was used as the baseline value, and the mean value of EPG counts on Day 28 ± 2 was used as the post-baseline value. The analysis of the efficacy criterion was performed on a log-transformed scale using an analysis of covariance adjusted for the baseline EPG counts.

Geometric means (GeoMeans) were calculated using the log-arithmetic mean (ArithMean) of the EPG counts of each animal. The GeoMean was calculated using the log-ArithMean of the EPG count of each animal, adding a “1” to the EPG count for each animal in both the Groups in view of the “zero” values of some EPG counts. This constant “1” was subtracted from the resultant calculated geometric mean prior to calculating percentage efficacy.

The difference between the GeoMeans for EPG before and after the treatment was expressed as percentage efficacy (%) using the following formula:

\[
\%\text{ Efficacy} = 100 \left( \frac{\text{GeoMean EPG at baseline} - \text{GeoMean EPG at post-baseline}}{\text{GeoMean at baseline}} \right)
\]

Treatment was deemed effective if a percentage reduction of at least 90% was achieved along with a significant difference (p < 0.05, two-sided) between the EPG counts in Group C and Group T.

3. Results

3.1. Dogs

All dogs were treated appropriately in accordance with the protocol, none were removed from the study subsequent to inclusion for any reason, and all were included in the efficacy calculations.

Seven out of the eight dogs in Group T were negative for C. boehmi eggs on Day 28 ± 2. The single positive dog received a second treatment and was examined again on Day 56 ± 2.

All eight animals in Group C were still infected on completion of the study, and seven received a rescue dose of Advocate® and were re-examined on Day 56 ± 2.

3.2. Efficacy evaluation

The ArithMean EPG count at baseline was 450 (±159.09) and 581.25 (±112.87) in Groups T and C respectively, with no significant difference in FECs between the two groups (p = 0.51).

The baseline EPG values in dogs in Group T were reduced from 450 (±159.09) to 48.12 (±48.12) on Day 28 ± 2 and to 0 on Day 56 ± 2, corresponding to an efficacy of 99.14 and 100% respectively (Table 1). Efficacy on Day 28 ± 2 (i.e. after a single administration of Advocate®) increased to 99.57% after data from the rescue treatment received by
seven control dogs were included. In Group C no evident difference between mean EPG before (581.2 ± 112.77) and after (584.37 ± 114.46) treatment was demonstrated, with a corresponding change of 1.74% from baseline.

The difference between Group T and Group C with regard to the change in EPG from baseline was 502.60 on Day 28 ± 2 and 504.70 on Day 56 ± 2, which is statistically significant (p < 0.01) (Table 1).

3.3. Clinical findings and outcome

Neither AEs nor SAEs were recorded in any of the treated dogs.

On Days -6 and -2 all the dogs in Group T and five in Group C showed various respiratory symptoms on clinical examination, i.e. repeated sneezing (n = 9 dogs), reverse sneezing (n = 2), nasal discharge (n = 4), epistaxis (n = 3), hypo-/anosmia (n = 3), cough (n = 4) and scratching of the nasal region (n = 1) (Table 2).

Clinical signs disappeared in seven of the eight symptomatic animals in Group T and in five animals which received the rescue treatment four weeks after the initial treatment had been given. The dog in Group T which received a second treatment was still symptomatic on Day 28 ± 2 but had fully recovered on Day 56 ± 2 (Table 3).

3.4. Rhinoscopy and molecular procedures

The presence of nasal capillariosis in animals which were positive for C. boehmi eggs at the faecal examination was confirmed by endoscopic and/or molecular approaches carried out at both pre- and post-treatment evaluations.

All dogs in Group T scored positive for adult stages of C. boehmi at rhinoscopy (Fig. 2) and/or for eggs following nasal flushing, whereas all the eight animals in Group C tested positive in molecular procedures applied to faecal samples where consent to the endoscopic procedure had not been given.

Post-treatment rhinoscopy was performed for six dogs in Group T because the owners of two dogs did not give their consent to additional anaesthesia. The negative result of copromicroscopy was confirmed by the aforementioned genetic assays for these two animals and for the control animals which received rescue treatment.

Of the eight dogs in Group T, seven were negative on endoscopy (n = 6) and in the confirmatory PCR (n = 1) conducted on Day 28 ± 2, while one dog that scored PCR-positive on Day 28 ± 2 was negative at the examination performed on Day 56 ± 2 (Table 3).

All seven dogs in Group C which were given rescue treatment scored negative on copromicroscopy and confirmatory PCR performed on Day 56 ± 2 (Table 3). Thus, a second administration of Advocate® was not necessary for these dogs.

---

**Table 1**

<table>
<thead>
<tr>
<th>Study period and statistics</th>
<th>T group</th>
<th>C group</th>
<th>Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline EPG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArithMean (SD)</td>
<td>450 (159.09)</td>
<td>581.2 (112.77)</td>
<td>F = 4.6, p = 0.51</td>
</tr>
<tr>
<td>95% CI</td>
<td>315/585</td>
<td>457.8/704.6</td>
<td></td>
</tr>
<tr>
<td>Min/Max</td>
<td>200/1525</td>
<td>275/1200</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>262.5</td>
<td>512.5</td>
<td></td>
</tr>
<tr>
<td>GeoMean</td>
<td>345.58</td>
<td>513.65</td>
<td></td>
</tr>
<tr>
<td>Post-baseline I EPG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArithMean (SD)</td>
<td>48.12 (48.12)</td>
<td>584.37 (114.46)</td>
<td>F = 4.6, p = 0.0007</td>
</tr>
<tr>
<td>95% CI</td>
<td>36.82/59.42</td>
<td>532.07–636.37</td>
<td></td>
</tr>
<tr>
<td>Min/Max</td>
<td>0/385</td>
<td>225/1125</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0</td>
<td>537.5</td>
<td></td>
</tr>
<tr>
<td>GeoMean</td>
<td>2.10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Percentage of efficacy (95% CI)</td>
<td>9.14% (95.3–99.9%)</td>
<td>3.17% (95% CI)</td>
<td></td>
</tr>
</tbody>
</table>

* Difference between groups from ANOVA adjusted for baseline.

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**Fig. 2.** Rhinoscopy: adult stages of Capillaria boehmi embedded in the mucosa of the middle meatus of a naturally infected dog in Group T.
Table 2
Clinical signs in sixteen dogs naturally infected with *Capillaria boehmi* at baseline.

<table>
<thead>
<tr>
<th>Group</th>
<th>Symptoms</th>
<th>None</th>
<th>Presence</th>
<th>Repeated sneezing</th>
<th>Reverse sneezing</th>
<th>Nasal discharge</th>
<th>Epistaxis</th>
<th>Hypo/Anosmia</th>
<th>Cough</th>
<th>Scratching of the nasal region</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>N. animals (%)</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>N. animals (%)</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>3 (18.75%)</td>
<td>9 (56.25%)</td>
<td>2 (12.5%)</td>
<td>11 (68.75%)</td>
<td>3 (18.75%)</td>
<td>3 (18.75%)</td>
<td>4 (25%)</td>
<td>1 (6.25%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group T: eight naturally infected dogs; Group C: eight control dogs left untreated.

Table 3
Pre- and post-treatment clinical findings and outcome, and positivity or negativity on rhinoscopy and molecular diagnosis in the 16 dogs included in the field trial.

<table>
<thead>
<tr>
<th>Group</th>
<th>ID</th>
<th>Day 28 ± 2</th>
<th>Day 56 ± 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>Rhinoscopy</td>
<td>PCR</td>
</tr>
<tr>
<td>T</td>
<td>1</td>
<td>+</td>
<td>+AW + NF</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>–AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>–AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>–AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+AW + NF</td>
<td>NP</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>NA</td>
<td>+</td>
</tr>
</tbody>
</table>

Group T: group treated; Group C: control group left untreated; ID: animal identification; CS: clinical signs; NA: not allowed; NP: not performed; +NF: positive nasal flushing for *C. boehmi* eggs; –NF: negative nasal flushing for *C. boehmi* eggs; +AW: positive for adult specimens of *C. boehmi*; –AW: negative for adult specimens of *C. boehmi*; +: positivity on rhinoscopy or molecular diagnosis; –: negativity on rhinoscopy or molecular diagnosis.

All sequences obtained from PCR amplicons generated from faeces proved to be the appropriate species *C. boehmi*, i.e. ~100% homology with sequences obtained from microscopically identified adult parasites.

4. Discussion and conclusion

This study, although preliminary, showed that Advocate® spot-on is safe and effective in the treatment of natural canine infection by *C. boehmi*.

The efficacy of this anthelmintic formulation was investigated on the basis of copromicroscopic results confirmed by rhinoscopy, nasal flushing or a molecular assay. A sensitivity <100% cannot be ruled out for the McMaster method. The negative results of copromicroscopy in treated dogs were therefore confirmed by the absence of adult parasites or eggs on rhinoscopy or nasal flushing, and of DNA in the PCR procedure. A similar PCR assay recently validated for pulmonary capillariosis due to *C. aerophila* may identify positive faecal samples which are negative on copromicroscopy (Di Cesare et al., 2012b). Thus, the negative results found in this study for parasitic DNA in the faeces may in fact signify the absence of *C. boehmi* in treated dogs.

The consistency between the McMaster method and the other approaches demonstrates that the former is a reliable approach to diagnosing the infection. Additionally, the diagnostic sensitivity of the test was assured by repeated quantitative examinations at each of the pre- and post-treatment sampling times.

At present there is no drug licensed for the treatment of *C. boehmi* infection in dogs, and the therapeutic regimens attempted have been derived empirically (Conboy, 2009; Baan et al., 2011; Veronesi et al., 2013). Moreover, some information derives from dated cases of nasal capillariosis in which a likely misidentification between *C. aerophila* and *C. boehmi* occurred (Evinger et al., 1985; King et al., 1990). The efficacy of benzimidazoles (BZs) and MLs has been described in single clinical cases, although the protocols used were not entirely satisfactory due to controversial or varying efficacy in treating clinical signs and stopping egg shedding.

The administration of fenbendazole at 50 mg/kg/day for ten consecutive days in a single dog produced fast recovery from clinical signs and assured negative faecal examinations at six weeks post-treatment (King et al., 1990). The same protocol led to the regression of clinical signs in a
dog which, however, was re-infected few weeks later. A second two-week course of fenbendazole was successful, and coprophagia was also prevented to avoid re-infection (Baan et al., 2011). While treatment with 0.5–1 mg/kg of milbemycin oxime was ineffective in the treatment of a dog with a history of chronic sneezing and intermittent post-exercise nasal discharge due to C. boehmi, a dosage of 2 mg/kg was effective, producing clinical recovery and cessation of faecal egg shedding (Conboy et al., 2013). The first evaluation of a single dose of 0.2 mg/kg ivermectin by the oral route demonstrated the clinical recovery of an infected dog and cessation of egg shedding for 8 and 4 months post-treatment, respectively (Evinger et al., 1985). However, the same treatment protocol also demonstrated an inconsistent reduction of clinical signs and the inability to eliminate faecal egg shedding, despite consecutive doses (King et al., 1990; Baan et al., 2011).

A recent report has described the usefulness of moxidectin (2.5 mg/kg) in inducing clinical recovery and negative faecal results, along with prevention of re-infection for four consecutive months, in a dog infected by C. boehmi (Veronesi et al., 2013). The present results confirm that a single administration of spot-on moxidectin is a suitable choice for the effective treatment of canine nasal capillariosis. In fact, in the present trial only one dog remained infected after a single administration of the molecule, although this did succeed in treating the parasitic infection after a second dose. Interestingly, this dog showed the highest pre-treatment EPG values in Group T, thus suggesting that two administrations are necessary to clear the infection in animals which may be heavily infected. Although preliminary, the present study has filled gaps in the knowledge of treatment of canine nasal capillariosis by evaluating moxidectin in a case series of infected dogs.

Apart from its high level of efficacy, Advocate® also has the advantages of single-dose administration and easy-to-apply topical delivery as compared to other molecules (e.g. fenbendazole) which require consecutive administrations.

In conclusion, Advocate® spot-on in dogs naturally infected by E. boehmi is a safe and effective option for treating clinical signs and eliminating egg shedding and adult parasites in situ. GCP studies are warranted to further evaluate the efficacy and safety of Advocate® in the therapy of canine infection with C. boehmi. These studies could also be worthwhile in terms of preventing the disease, given that infected dogs are at high and frequent risk of recurrent infections (King et al., 1990; Baan et al., 2011; Veronesi et al., 2013).

Conflicts of interest

Bayer Animal Health GmbH, Germany, provided financial support for this study. The Authors declare that there were no competing interests and that the conceptual design, the conduct, the interpretation of results and all scientific aspects of the study were not influenced by any third party.

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