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Original Article

Evaluation of the Clinical Efficacy and Safety of a Spot-on Combination of Imidacloprid 10% / Moxidectin 2.5% (Advocate®, Advantage® Multi) in Comparison to an Untreated Control Group in the Treatment of *Capillaria boehmi* in Naturally Infected Dogs

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Abstract

Capillaria boehmi affects the upper respiratory tract of domestic and wild canids. The aim of the present study was to investigate the efficacy of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate®, Advantage® Multi, Bayer) in dogs naturally infected by *C. boehmi*. Twenty dogs infected with *C. boehmi* were randomly allocated to two groups: T1 (10 dogs) received a single treatment of Advocate® using the recommended dose on day 0 and T2 (10 dogs) served as an untreated control group. The reduction of the faecal egg counts (EPG) from baseline (days -6±2 and -2±2) to study completion was set as the primary efficacy criterion; clinical assessments of the upper respiratory tract and a rhinoscopy to visualize the parasites were used as secondary efficacy criteria. Eight dogs in T1 were not shedding eggs on days 28±2 (reduction of EPG 99.66%). A second treatment was administered to two dogs still positive on days 30±3. A second efficacy evaluation was performed on days 42±2 (study completion), when the two dogs tested negative. The mean number of EPG at study completion was 0 in T1 and 368.49 in T2. The difference between the groups was statistically significant ($P < 0.01$). Treatment efficacy at study completion was 100%. None of the T1 dogs showing clinical signs on day 0 were symptomatic on days 28±2. No adverse events occurred. The results show that Advocate® is safe and effective in the treatment of canine nasal capillariosis.

Introduction

Nasal capillariosis is a respiratory disease caused by *Capillaria boehmi* (syn. *Eucoleus boehmi*), a trichuroid nematode affecting the epithelium of the nasal turbinates, frontal and paranasal sinuses of wild canids (e.g. foxes) and domestic dogs (Conboy 2009). Knowledge of the biological cycle, range of hosts and epidemiology of nasal capillariosis is still fragmentary (Campbell and Little 1991, Conboy 2009) but it is assumed that animals acquire the infection by ingesting larvated eggs from the environment and/or infected earthworms that could act as facultative intermediate or paratenic hosts (Conboy 2009). The infection has been described in dogs from North America (reviewed in Veronesi et al. 2014a), South America (González et al. 2014) and Europe (reviewed in Veronesi et al. 2014a, Alho et al. 2016). An increased prevalence of the disease in dogs has been related to higher exposure in areas where the natural reservoirs, mainly foxes, are present due to human expansion into wildlife areas (Piperisova et al. 2010, Veronesi et al. 2014b). Dogs infected by *C. boehmi* may be subclinically infected or show upper respiratory signs of varying severity, i.e. sneezing, reverse sneezing, hypo- or anosmia and catarrhal blood-stained or muco-purulent nasal discharge when bacterial infection occurs (Evinger et al. 1985, Campbell and Little 1991, Piperisova et al. 2010, Veronesi et al. 2013). Meningoencephalitis and generalized convulsive seizures have been observed as a consequence of aberrant migration into the cranial cavity (Clark et al. 2013).

Capillaria boehmi is doubtless underestimated, probably due to the lack of knowledge about this nematode among veterinarians, the occurrence of unspecific clinical signs and the difficulties in achieving an aetiological diagnosis (Di Cesare et al. 2015). In clinical settings the diagnosis relies on standard faecal flotations for detecting the typical eggs, which need to be differentiated from those of the lungworm *Capillaria aerophila* and of the best-known canine intestinal whipworm *Trichuris vulpis* (Di Cesare et al. 2012). The detection of adult worms and eggs during rhinoscopic examination or cytologic evaluation of nasal flushing may also be used to diagnose the infection (Baan et al. 2011, Veronesi et al. 2013). Nevertheless, rhinoscopy is expensive, invasive, requires anaesthesia and is unsuitable when the parasite is located in the caudal portion of the nasal cavity or when abundant mucus is present (Veronesi et al. 2014a). Finally, a species-specific molecular assay which enables identification of DNA of *C. boehmi* from faecal samples has recently been developed, though not used in routine clinical practice (Di Cesare et al. 2015).

Despite the fact that *C. boehmi* may play a pathogenic role in dogs, no products are licensed in Europe for treating the infection. A pilot trial carried out in 2012-2013 demonstrated the high efficacy (99.57 %) and safety of a single application of a spot-on formulation containing imidacloprid 10 % / moxidectin 2.5 % (Advocate®) in the treatment of the infection (Veronesi et al. 2014a). The present article describes a good clinical practice (GCP) trial designed to further evaluate the clinical efficacy and safety of Advocate® in comparison to an untreated control group in dogs naturally infected by *C. boehmi*.

Materials and methods

The study was a negative-controlled, multicentric clinical field study carried out in two veterinary practices located in central Italy (see acknowledgements) from February to July 2016. The study was conducted according to European and national regulatory requirements and in compliance with the following guidelines:

- Directive 2001/82/EC as amended
- VICH GL9 (Good Clinical Practice, June 2000)
- VICH GL7: Efficacy of anthelmintics: General requirements (CVMP/VICH/832/99-corr)
- VICH GL19: Efficacy of anthelmintics: Specific recommendations for canines

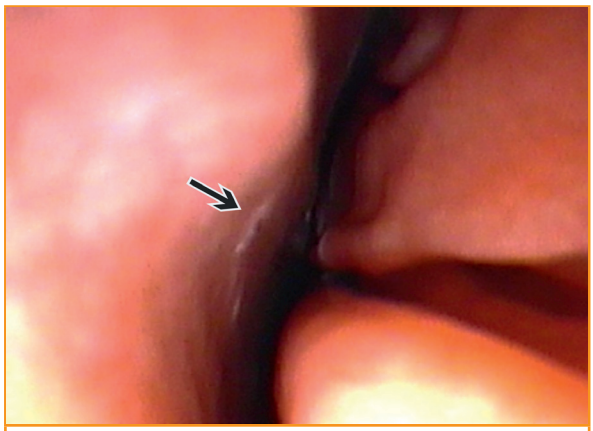

Fig. 1 Dog C101A, day 0, rhinoscopic examination. Presence of an adult specimen of *Capillaria boehmi in situ* (arrow)

- Directive 81/852/EEC
- EMA/CVMP/EWP/81976/2010: Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals)

Enrolment, study inclusion, clinical examinations and treatment

Twenty dogs found to be infected with *C. boehmi* by the detection of eggs in faecal samples using a coproscopic concentration-flotation procedure (Sloss et al. 1994) were selected. Eggs of *C. boehmi* were identified on the basis of the typical morphological and morphometric features (Di Cesare et al. 2012) during a screening phase. Dogs were subjected to a physical and clinical examination and the owner's written consent was provided for each animal.

Two faecal samples were collected from each *C. boehmi*-infected dog between days -6 ± 2 and -2 ± 2. Each individual faecal sample was processed with two quantitative McMaster technique flotations, one using a sugar solution with a specific gravity (s.g.) of 1.200, the other using a zinc sulphate solution with an s.g. of 1.350, to confirm the infection and to determine the baseline numbers of eggs per gram faeces (EPG). A pre-treatment clinical examination was performed to detect clinical signs related to nasal capillariosis.

On day 0 all dogs which scored positive for eggs of *C. boehmi* at the quantitative coproscopic examinations were submitted to a rhinoscopy to detect the presence of the adult parasite *in situ*. Anterior and posterior rhinoscopic examinations were performed using a single flexible endoscope of appropriate size. Direct visualization of the parasite was assigned to 2 different classes of intensity (Class 1: 1 to 3 adult parasites and Class 2: more than 3 adult parasites). Results of the rhinoscopy were regarded as positive if at least one adult *C. boehmi* could be visualized in the nasal cavities (Fig. 1).

Animals meeting the following criteria were included in the study:

- Coproscopic detection of eggs of *C. boehmi*
- Dogs with satisfactory general health on physical examination
- Signed owner informed consent form

Animals meeting the following criteria were not included in the study:

- Dogs which had received macrocyclic lactones or any other anthelmintic with a systemic biodistribution during the two months before the study started
- Dogs affected by concomitant parasitic respiratory infections
- Dogs younger than 7 weeks and weighing less than 1 kg
- Pregnant or lactating dogs
- Dogs with known hypersensitivity to at least one of the ingredients of the product
- Dogs with a known infection with *Dirofilaria immitis*, class 4

The twenty dogs were randomly allocated in a 1:1 ratio to two study groups, i.e. Group T1 (n = 10 dogs) treated with Advocate® and Group T2 (n = 10 dogs) left untreated.

Dogs in Group T1 were treated topically on day 0 with a single dose of Advocate®, according to body weight (BW) and label instructions (Table 1). A second treatment was planned for those animals

Table 1 Weights of dogs enrolled in T1, dosage of Advocate® (imidacloprid 10 % and moxidectin 2.5 %) applied and dosage of moxidectin actually administered in terms of mg/kg body weight (b.w.).

Animal ID	Study day	Body weight kg	Dosage applied of Advocate®	Moxidectin mg/kg b.w.
C101A	0	22.1	>10–25 kg Advocate® for large dogs (250 mg imidacloprid 62.5 mg moxidectin)	2.83
	32	22.3		2.80
C104A	0	22.9		2.73
C104B	0	15.4		4.06
C105A	0	17.2		3.63
C105B	0	16.1		3.88
D102A	0	19.6		3.19
D102B	0	21.4		2.92
D103A	0	13.2		4.73
D103B	0	16.2		3.86
	32	15.9		3.93
D103C	0	20.2		3.09

Table 2 Mean number of egg per gram of faeces (EPG) and percentage reduction (% reduction) of *Capillaria boehmi* from baseline to study completion.
T1: dogs treated with Advocate®.
T2: dogs left untreated. Out of 10 dogs allocated to T1, 8 completed the study on days 28±2 while 2 were treated again on days 30±3 and completed the study on days 42±2.

	EPG			
	T1		T2	
	Baseline	Completion	Baseline	Completion
Geometric mean	392.81	0.00	233.37	368.49
Minimum	75.00	0.00	25.00	100.00
Maximum	900.00	0.00	775.00	950.00
	% Reduction			
	T1		T2	
Geometric mean	100.00		No reduction	
95 % CI lower limit	100.00			
95 % CI upper limit	100.00			

that were still positive for *C. boehmi* on days 28±2 (see following section) (Table 1).

All treated animals were physically examined within 3–5 hours post-treatment to evaluate the safety and the potential side effects of the spot-on formulation administered. Dogs belonging to the untreated control group (T2) received a rescue treatment of Advocate® after completion of the study.

Post-treatment evaluation

Twenty-four hours prior to days 28±2, two faecal samples were collected to perform a follow-up EPG. Each individual faecal sample was processed as above. A clinical examination and a post-treatment rhinoscopy were performed on days 28±2. If EPG and rhinoscopy were negative, dogs in both groups completed the study on days 28±2. Dogs

in T1 with a positive result on days 28±2, either in EPG or rhinoscopy, were treated with a second dose of Advocate® according to body weight and label instructions on days 30±3, while positive T2 dogs were left untreated.
Dogs in both groups which tested positive on days 28±2 underwent coproscopic tests of two faecal samples as described above and a clinical examination on days 42±2.

Data analysis

For each individual faecal sample (i.e. two pre-treatment samples collected between days -6±2 and -2±2) and post-treatment samples collected on days 28±2 and 42±2 the "Quantitative Result" was calculated as the mean of the faecal egg counts from the two different flotation procedures.
The primary efficacy criterion was the reduction of the EPG from baseline to the study completion visit, calculated as % reduction according to the formula:

$$\text{Reduction (\%)} = 100 \times \frac{\text{Mean EPG at baseline} - \text{Mean EPG at study completion}}{\text{Mean EPG at baseline}}$$

The EPG at baseline was the higher "Quantitative Result" from the two faecal samples collected between days -6±2 and -2±2. Analogously, the EPG at study completion was the higher "Quantitative Result" from the two faecal samples collected prior to study completion.
Mean EPG was calculated as the geometric mean.
The study was completed when the animal turned negative, either on days 28±2 in the case of a negative coproscopic and/or rhinoscopic result, or on days 42±2 in the case of a positive coproscopic or rhinoscopic result on days 28±2.

The secondary efficacy criteria were:

- The reduction of the faecal egg count of *C. boehmi* from baseline to day 28 (post-baseline collection I) that was calculated as described above for the primary efficacy criterion
- The reduction of the faecal egg count of *C. boehmi* from baseline to day 42 (post-baseline collection II) that was calculated as described above for the primary efficacy criterion
- The presence of adult stages of *C. boehmi* determined by rhinoscopy on days 28±2
- The presence of clinical signs of nasal capillariosis on days 28±2 and on days 42±2, each compared to baseline

Results

Dogs

All dogs were treated in accordance with the protocol, none was removed from the study for any reason, and all were included in the efficacy calculations.
Eight dogs (i.e. 8/10, 80 %) allocated to T1 completed the study on days 28±2 due to negative coproscopic and rhinoscopic results. Two T1 animals were treated again on days 30±3 due to a positive coproscopic result (1 dog) or a positive rhinoscopic result (1 dog) on days 28±2. Both were negative on days 42±2.
All ten animals allocated to T2 completed the study on days 42±2 due to a positive coproscopic result for *C. boehmi* in post-baseline collection I (i.e. days 28±2).

Reduction of EPG at study completion

The geometric mean number of EPG on the day of study completion was 0 in the T1 group and 368.49 in the untreated T2 group (Table 2); the difference between groups was statistically significant (P=0.0004). The percentage efficacy (% reduction of EPG from baseline) at study completion in the T1 group was 100 %. The mean number of EPG showed an increase in EPG from baseline to study completion in the T2 group (Table 2).

Reduction of EPG at post-baseline collection I and II

The geometric mean number of EPG at post-baseline collection I (days 28±2) was 0.54 in the T1 group and 299.50 in the T2 group. In the T1 group the reduction of the faecal egg count on

Table 3 Percentage reduction of number of faecal egg counts of *Capillaria boehmi* from baseline to post-baseline collection I (days 28 ± 2) and post-baseline collection II (days 42 ± 2).
T1: dogs treated with Advocate®. T2: dogs left untreated.

	Post-baseline collection I		Post-baseline collection II	
	T1	T2	T1	T2
Geometric means	99.86 %	No reduction	100.00 %	No reduction
95 % CI lower limit	99.16 %		100.00 %	
95 % CI upper limit	100.00 %		100.00 %	

Table 4 Presence of adult stages of *Capillaria boehmi* in T1 (dogs treated with Advocate®) and T2 (dogs left untreated) on day 0 (treatment day) and +28 ± 2 (post treatment evaluation).
Class 1 = 1 to 3 adult parasites; Class 2 = more than 3 adult parasites. n.d. = not done

		T1 (n = 10)	T2 (n = 10)	Fisher's test
Presence of adult stages of *C. boehmi*				
Day 0	No	5 (50.00 %)	8 (80.00 %)	P = 0.350
	Yes	5 (50.00 %)	2 (20.00 %)	
Days 28 ± 2	No	9 (90.00 %)	9 (90.00 %)	P = 1.0
	Yes	1 (10.00 %)	1 (10.00 %)	
Classification				
Day 0	Class 1	4 (40.00 %)	2 (20.00 %)	n.d.
	Class 2	1 (10.00 %)	0 (0.00 %)	
Days 28 ± 2	Class 1	1 (10.00 %)	1 (10.00 %)	

Table 5 Presence of clinical signs in dogs allocated to T1 (dogs treated with Advocate®) and T2 (dogs left untreated) on day 0, +28 ± 2 and 42 ± 2

Clinical signs	Group	Day 0 n (%)	Day 28 ± 2 n (%)	Day 42 ± 2 n (%)
Sneezing	T1	4 (40.00 %)	0	0
	T2	3 (30.00 %)	6 (60.00 %)	6 (60.00 %)
Reverse sneezing	T1	2 (20.00 %)	0	0
	T2	3 (30.00 %)	3 (30.00 %)	4 (40.00 %)
Nasal discharge	T1	5 (50.00 %)	0	0
	T2	5 (50.00 %)	7 (70.00 %)	7 (70.00 %)
Hypo-/anosmia	T1	3 (30.00 %)	0	0
	T2	2 (20.00 %)	2 (20.00 %)	2 (20.00 %)
Cough	T1	2 (20.00 %)	0	0
	T2	1 (10.00 %)	1 (10.00 %)	1 (10.00 %)

days 28±2 was 99.86% (95% CI 99.16; 100.00) with a lower limit of the 95% CI greater than 90% (Table 3). The geometric mean number of EPG at post-baseline collection II (days 42±2) was 0.00 in the T1 group and 368.49 in the T2 group. The treatment was 100% effective on days 42±2 (Table 3).

Rhinoscopy and clinical evaluation

At none of the examination time points was the number and percentage of animals with adult stages of *C. boehmi* significantly different between the T1 and T2 groups (P >0.3) (Table 4). Due to the low absolute numbers, the classification of adult stages of *C. boehmi* was evaluated descriptively without statistical testing (Table 4).

On day 0 the presence of sneezing, reverse sneezing, nasal discharge, hypo-/anosmia and cough was equally distributed between the animals allocated to T1 and T2 (Table 5). None of the T1 dogs that showed clinical signs on day 0 were symptomatic on days 28±2 and 42±2, thus indicating a 100% clinical recovery. In group T2 the percentage of animals with nasal discharge increased from 50% on day 0 to 70% on days 28±2 and 42±2, the incidence of sneezing doubled from 30% on day 0 to 60% on days 28±2 and 42±2 and the number of animals showing reverse sneezing increased from 30% on days 28±2 to 40% on day 42±2. On days 28±2 nasal discharge and sneezing were significantly more frequent on T2 dogs (Table 5).

No adverse events were recorded in any of the treated dogs.

Discussion

The present study demonstrated the high efficacy of Advocate® spot-on solution in the treatment of nasal capillariosis in naturally infected dogs. The high level of faecal egg output reduction was confirmed by the absence of adult parasites or eggs upon rhinoscopy at study completion. The full recovery of all dogs with respiratory signs following the first treatment with Advocate® further supports the evidence of a high efficacy of the treatment against the parasite. These data are corroborated by the increasing percentage of T2 dogs showing clinical signs on days 28±2 compared to baseline.

Overall, the data presented here confirmed previous findings achieved in a recent pilot trial that showed a reduction of post-baseline egg counts by 99.57% after a single application of the same topical combination (Veronesi et al. 2014a). These results are of importance in view of the fact that no drugs are currently marketed for use in dogs infected with *C. boehmi*, and that the vast majority of treatment protocols are limited to a few number of animals and based on empirical observations.

An older publication describes the recovery of clinical signs and a negative faecal examination in a dog after treatment with fenbendazole administered for 10 days at 50 mg/kg BW/day (King et al. 1990). More recently, it has been suggested that the infection can be successfully treated with a two-week course of fenbendazole (50 mg/kg BW per os) combined with removing faeces from the dog's environment to prevent reinfection (Baan et al. 2011). In another study, a single dose of oral ivermectin (0.2 mg/kg BW) achieved the clinical recovery of an infected dog and cessation of egg shedding (Evinger et al. 1985). Repeated treatments with 0.5–1 mg/kg BW of milbemycin oxime were ineffective in treating nasal capillariosis in a symptomatic dog, while the same macrocyclic lactone, at a dosage of 2 mg/kg, achieved the cessation of faecal egg shedding (Conboy et al. 2013). Nevertheless, a single administration of 2 mg/kg BW of milbemycin oxime was not effective in another recent case from Portugal (Alho et al. 2016). This dog received a second unsuccessful treatment for 2 weeks with fenbendazole (100 mg/kg BW per os) along with preventive measures, but the infection was not resolved until a final single administration of Advocate® had been given (Alho et al. 2016). The present data, along with other recent information (Veronesi et al. 2013, 2014a, Alho et al. 2016),

confirm that moxidectin used in a spot-on formulation is a suitable choice for effective treatment and control of canine nasal capillariosis. Taking into account the potential direct life cycle of *C. boehmi*, further measures such as prompt removal of faeces from the environment and prevention of coprophagia or geophagic pica should be taken to prevent reinfection. At the same time, post-treatment faecal examinations should also be performed to monitor the efficacy of the anthelmintic therapy and the occurrence of reinfection (Baan et al. 2011, Veronesi et al. 2013, Alho et al. 2016).

The repeated monthly administration of the molecule has been shown to result also in a steady state of the product (i.e. a sustained, elevated plasma level of moxidectin) in treated dogs, and the same phenomenon is documented for the feline product (Cruthers et al. 2008, Little et al. 2015). Thus, the elevated plasma levels of moxidectin may have the potential to prevent circulating larval stages from developing into adult worms. Further studies are warranted in at-risk categories of dogs (e.g. dogs living in areas contaminated by the eggs of *C. boehmi*) to investigate the potential of moxidectin in protecting dogs from nasal capillariosis.

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of the institution or practice at which the study was conducted. This article does not contain any studies with human participants performed by any of the authors.

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Conflict of Interest

The authors Fabrizia Veronesi, Angela Di Cesare, Gabriele Braun, Lisa Günther, Giulia Morganti, Fabrizio Rueca and Donato Traversa declare that they have no conflict of interest. Gabriele Petry and Roland Schaper are employees of Bayer Animal Health GmbH which sponsored the present study.

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