Effectiveness of the spot-on combination of moxidectin and imidacloprid (Advocate®) in the treatment of ocular thelaziosis by *Thelazia callipaeda* in naturally infected cats

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**Abstract**

**Background:** The present study evaluated the therapeutic effectiveness of moxidectin 1.0% (w/v) and imidacloprid 10% (w/v) (Advocate® spot-on solution for cats, Bayer Animal Health) against natural infections with the eyeworm *Thelazia callipaeda* in cats. This study was conducted as a GCP, negative-controlled, blinded and randomised field study in privately owned cats living in an area in southern Italy where *T. callipaeda* is enzootic.

**Methods:** The study was carried out in 30 cats (19 females and 11 males, aged from 8 months to 5 years, weighing 1.2–5.2 kg) of different breeds, naturally infected by *T. callipaeda*. At study inclusion (Day 0), animals were physically examined and the infection level was assessed by examination of both eyes for clinical score and live adult *T. callipaeda* count. Each cat was weighed and randomly assigned to one of the treatment groups (G1: Advocate, G2: untreated control). Clinical assessments and *T. callipaeda* adult counts were performed on Day 14. At the study completion visit on Day 28, clinical assessments and counts of *T. callipaeda* adults and larvae were performed. All cats were daily observed by their owners and general health conditions were recorded during the entire period of the study.

**Results:** The primary effectiveness variable was the percentage of animals in G1 group (Advocate) showing a complete elimination (parasitological cure) of adult eye worms at Day 14 and Day 28. The effectiveness of the treatment in the G1 group was 93.3 and 100% at Day 14 and Day 28, respectively, when compared to group G2. Total worm count reduction from both eyes for Advocate was 96.3% on Day 14 and 100% on Day 28. Clinical data were confirmed by the examination of conjunctival pouch flushing. An overall reduction in the number of cats with lacrimation and conjunctivitis was observed following treatment despite the fact that in a few cats treated with Advocate clinical signs persisted due to the chronic nature of the disease.

**Conclusions:** Based on the results of the present trial, a single dose of Advocate was found to be safe and highly effective in the treatment of natural *T. callipaeda* infection in cats.

**Keywords:** Advocate, Eyeworm, Zoonosis, Moxidectin, Parasite, *Thelazia callipaeda*, Treatment
Background

*Thelazia callipaeda* is a spirurid nematode residing in the conjunctival pouches of dogs, cats, wild carnivores and humans. Since its first description in dogs from northern Italy [1], reports of thelaziosis have increased showing a wide distribution throughout European countries and its capability to provoke ocular manifestations such as conjunctivitis, keratitis, corneal opacity and ulcers [2–4] due to the mechanical action of the adult parasite on the conjunctival and corneal epithelium. Due to its original distribution in East Asian countries, this worm used to be regarded as the “oriental eye-worm”, but after more recent descriptions in animals and humans throughout European countries such as Italy [5], Spain [6, 7], Croatia [8], Greece [9], Serbia [10], Bulgaria and Hungary [11], this term is no longer appropriate. The increase of infections in both animals and humans is related to the presence of the *T. callipaeda* vector, *Phortica variegata*, a drosophilid of the subfamily Steganinae which, in Europe, acts as intermediate host of the eyeworm [12, 13].

*Phortica variegata* live in forested and meadow areas where several competent wild hosts (e.g. red foxes, wolves, beech martens and wild cats [14]) perpetuate the infection, being reservoirs of the infection to dogs and cats [15]. Among available treatment procedures, physical removal of nematodes from conjunctival pouches consisting in a saline rinse (effective for adult and immature nematodes) and mechanical adult nematode collection by fine forceps or saline rinse (effective for adult and immature nematodes) of nematodes from conjunctival pouches consisting in a saline rinse (effective for adult and immature nematodes) and mechanical adult nematode collection by fine forceps or swabs has been described [16]. For canine thelaziosis, many treatments have been proposed, including topical instillation of organophosphates [17] or moxidectin [18]; however, these had major local side effects.

In 2016, Otranto et al. [19] published results on the effectiveness of moxidectin 2.5% w/v and imidacloprid 10% w/v (Advocate® spot-on solution for dogs, Bayer Animal Health) in thelaziosis showing a high effectiveness: a single application of this drug was sufficient to safely eliminate 100% of nematodes in infected dogs within seven days after a single administration. Moreover, the latter formulation acts systemically after topical application and consequently ocular irritation as local side effect while treating eye worm infections will not occur. In cats, the administration of milbemycin oxime at 2 mg/kg was demonstrated to have a high effectiveness in the treatment of *T. callipaeda* infections [20], but no registered products are currently available for the treatment of thelaziosis in this species.

The aim of this study was to evaluate the effectiveness of moxidectin 1.0% w/v and imidacloprid 10% w/v (Advocate spot-on solution for cats, Bayer Animal Health) against natural *T. callipaeda* infection in cats.

Results

At baseline all cats were infected by *T. callipaeda*. The average number of adult *T. callipaeda* specimens observed in G1 and G2 was 2.13 and 1.87, respectively. The number of parasites and the number of cats with clinical manifestations (lacrimation, conjunctivitis and ocular discharge; Table 1) in each eye was homogenous among groups (\(F_{(1,28)} = 0.767, P = 0.389\) and Fisher’s exact test, \(P = 0.500\), for the number of parasites and for lacrimation, conjunctivitis and ocular discharge, respectively). Keratitis and ulcers were absent in all the cats. The number and percentage of cats positive for *T. callipaeda* infection at each study day are reported in Table 2. Effectiveness in G1 (Advocate) was 93.3% on Day 14 and 100% on Day 28. The total number and mean of live *T. callipaeda* adults retrieved and counted on both eyes is reported in Table 2. The reduction in the number of worms counted in both eyes was 96.3% on Day 14 and 100% on Day 28 for the treated group. A natural mild decrease was observed for the untreated group showing a worm reduction of 3.6% on Day 14 and 7.1% on Day 28. The comparison of worm reduction between groups showed a significant difference at all post-treatment visits when tested by ANOVA (\(F_{(1,28)} = 67.600, P < 0.0001\)). Among ocular signs associated with *T. callipaeda* presence, only lacrimation, conjunctivitis and discharge were recorded in the included cats; details of observed symptoms are reported in Table 1. None of the cats exhibited keratitis and ulcers except one cat in the untreated group that showed both symptoms at the study closure visit (Day 28). A significant difference between treated and control groups were observed for lacrimation on Day 28.

| Symptom       | D0       | D14      | D28      |
|---------------|----------|----------|----------|
| Lacrimation   |          |          |          |
| G1            | 10 (66.6)| 5 (33.3) | 5 (33.3) |
| G2            | 9 (60.0) | 10 (66.6)| 11 (73.3)|
| Conjunctivitis|          |          |          |
| G1            | 3 (20.0) | 1 (6.7)  | 2 (13.3) |
| G2            | 4 (26.7) | 3 (20.0) | 8 (53.3) |
| Discharge     |          |          |          |
| G1            | 1 (6.7)  | 0 (0)    | 0 (0)    |
| G2            | 0 (0)    | 0 (0)    | 2 (13.3) |
| Keratitis     |          |          |          |
| G1            | 0 (0)    | 0 (0)    | 0 (0)    |
| G2            | 0 (0)    | 0 (0)    | 1 (6.7)  |
| Ulcers        |          |          |          |
| G1            | 0 (0)    | 0 (0)    | 0 (0)    |
| G2            | 0 (0)    | 0 (0)    | 1 (6.7)  |

Abbreviations: G1 treated at D0 with Advocate, G2 untreated control, D study day
\(aP = 0.028\)
\(bP = 0.020\)
28 (Fisher’s exact test, \( P = 0.028 \)) and for conjunctivitis on Day 28 (Fisher’s exact test, \( P = 0.020 \)). No treatment related adverse effects were recorded.

**Discussion**

A single spot on application of Advocate, containing moxidectin 1.0% (w/v) and imidacloprid 10% (w/v), was highly effective in the treatment of feline thelaziosis. Only one cat out of 15 treated was found positive two weeks after treatment and no worms were detected four weeks after treatment administration. No side effects were observed during the whole study period. As was previously demonstrated for dog patients [21], this commercial formulation showed to be safe and effective for the treatment of *T. callipaeda* infection in feline patients. In this study a low prevalence of ocular clinical signs was observed, confirming that the disease has a high variability in the clinical presentation and that the occurrence of subclinical asymptomatic infections is common. Even if some clinical signs showed a significant reduction (lacrimation and conjunctivitis) in treated cats, the observation of the persistency of clinical signs in a few treated animals, also following parasitological cure is due to the nature of the disease that frequently has a chronic course. Concerning the “One Health” approach, as reported elsewhere [15], cases of human thelaziosis are reported in areas where the infection is highly prevalent in the animal population. Cats are hosts that can often reach high densities in peridomestic habitats; therefore, a safe and effective drug being approved for the treatment of feline thelaziosis represents an important asset in *T. callipaeda* control.

**Conclusions**

The results of the present study demonstrate that Advocate is safe and highly effective in the treatment of *T. callipaeda* infection in cats after single administration of the recommended dose rate.

**Methods**

The study was conducted as a Good Clinical Practice (GCP), negative controlled, blinded and randomized field study in privately owned cats living in a *T. callipaeda* enzootic area of the Basilicata region (southern Italy), following approval by the Italian Ministry of Health (DGSAF- 0028002-06/12/2016-DGSAF-MDS-P). Study animals were located in areas enzootic for thelaziosis where the presence of the vector and of the diseases in dogs and cats was already proven [20, 22]. A sample size of 30 cats, 15 per group, was estimated to detect a difference between proportions (i.e. % of non-infected cats) in treated animals versus untreated, assuming a prevalence of infected cats in the untreated group of 73.3% (Motta et al. [20]) and a confidence level of 95% [software nQuery+nTerim 3.0 (StatSols, Statistical Solutions© Ltd. 2014)]. Thirty cats (19 females and 11 males) aged from 8 months to 5 years, of different breeds, in good health conditions and with at least one live adult *T. callipaeda* nematode in one eye (Fig. 1) were included in the study following the collection of the owner informed consent form. Details are provided in Table 3.

At inclusion (D0) animals were physically examined, weighed and inspected at both eyes, including a thorough examination underneath the third eyelid to retrieve and count live adult *T. callipaeda*. Clinical manifestations suggestive of eye worm infection such as lacrimation, conjunctivitis, ocular discharge, keratitis, and ulcers, were recorded and classified as absent, mild, moderate or severe.

Allocation to study groups (G1: Advocate, G2: untreated) was done in accordance with a random treatment allocation plan. In order to avoid bias due to the contact between treated and untreated cats, animals of the same household were allocated to the same study group. Advocate was administered on the basis of the body weight, following the instructions reported on the label of the commercial product.
Effectiveness (%) in the treatment of *T. callipaeda* infection was calculated for each time point using the following formula:

\[
\text{Effectiveness (\%)} = \left( \frac{\text{Positive animals in the untreated group} - \text{Positive animals in the treated group}}{\text{Positive animals in the untreated group}} \right) \times 100
\]

Fisher's exact test calculated on contingency tables for parasitological cure with 5% significance level of probability of rejecting the null hypothesis.

Worm count and ocular clinical signs reductions in the treated group were compared at time points (t) Day 14 and Day 28 with the untreated group as follows:

\[
\%\text{reduction } [t] = \left( \frac{C_{t0} - C_t}{C_{t0}} \right) \times 100,
\]

where *C*<sub>t0</sub> was the baseline count before treatment and *C*<sub>t</sub> was the count at time *t* after treatment for each secondary variable under investigation. The significance of the worm count reduction in treated cats was analyzed by ANOVA with standard statistical assumption. Statistical analysis was planned and conducted in compliance with current guidelines [25]. Statistical calculations and randomization were performed with: SPSS® statistical package for Windows, v.23.0 and nQuery + nTerim 3.0 (StatSols).

### Abbreviations

- **G1**: treated at D0 with Advocate, G2 untreated control
- **Female/Male**
- **Long/Medium/Short**

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**Table 3** Description of the characteristics of the cats included in the study. Ranges for age and weight are reported in parentheses.

|                  | G1 (n = 15) | G2 (n = 15) | Total (n = 30) |
|------------------|-------------|-------------|---------------|
| Age (months)     | 20.7 (8–60) | 23.5 (9–48) | 22.1 (8–60)   |
| Sex (no. of cats)<sup>a</sup> | 12/3        | 7/8         | 19/11         |
| Weight (kg)      | 2.7 (1.2–5.2) | 2.8 (1.2–4.3) | 2.8 (1.2–5.2) |
| Hair length (no. of cats)<sup>b</sup> | 0/14/1      | 0/12/3      | 0/26/4        |

**Abbreviations:** G1 treated at D0 with Advocate, G2 untreated control
<br><sup>a</sup>Female/Male
<br><sup>b</sup>Long/Medium/Short
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