



Research paper

Efficacy of moxidectin 2.5% and imidacloprid 10% in the treatment of ocular thelaziosis by *Thelazia callipaeda* in naturally infected dogs

Domenico Otranto^{a,*}, Vito Colella^a, Giuseppe Crescenzo^a, Fabrizio Solari Basano^b, Roberto Nazzari^b, Gioia Capelli^c, Gabriele Petry^d, Roland Schaper^d, Matthias Pollmeier^d, Egidio Mallia^e, Filipe Dantas-Torres^{a,f}, Riccardo Paolo Lia^a

^a Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, 70010 Valenzano, Italy

^b Arcoblu s.r.l., Via Alessandro Milesi 5, 20133 Milano, Italy

^c Istituto Zooprofilattico Sperimentale delle Venezie, 35020 Legnaro, Italy

^d Bayer Animal Health GmbH, 51368 Leverkusen, Germany

^e Parco Regionale Gallipoli Cognato e Piccole Dolomiti Lucane, 75011 Accettura, Italy

^f Department of Immunology, Aggeu Magalhães Research Centre, Oswaldo Cruz Foundation, 50670-420 Recife, Brazil

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ABSTRACT

Thelazia callipaeda (Spirurida, Thelaziidae) has been documented as agent of ocular infection in domestic animals (dogs and cats), wildlife (e.g., foxes, hares, rabbits), and humans. In the last two decades, this parasitosis has been increasingly reported in several European countries. Both adult and larval stages of the eyeworm are responsible for symptoms ranging from mild (e.g., lacrimation, ocular discharge, epiphora) to severe (e.g., conjunctivitis, keratitis, and corneal opacity or ulcers). The present study evaluated the clinical efficacy and safety of imidacloprid 10% and moxidectin 2.5% spot on (Advocate[®], Bayer Animal Health) in comparison to milbemycin oxime/praziquantel tablets (Milbemax[®], Novartis-Animal Health), as positive control, in the treatment of canine thelaziosis in naturally infected dogs and, a third group was used as an untreated control. Forty-seven dogs (27 females and 20 males) harbouring at least one live adult worm of *T. callipaeda* in one eye were enrolled from an endemic area of southern Italy. Each dog was then weighed and assigned in accordance with a random treatment allocation plan to one of the treatment groups (G1: imidacloprid 10% and moxidectin 2.5% spot on, G2: Untreated control and G3: milbemycin oxime/praziquantel tablets). On Day (D) 7, 14, 28 and 35 dogs were physically examined and the infection level was assessed by examination of both eyes, including conjunctival pouch and third eyelid for live adult *T. callipaeda* count and clinical scores. Dogs in G1 were treated on D0 and D28, whereas those in G3 on D0 and D7. Efficacy in G1 was 100% at each day post treatment ($p < 0.01$). For the G3 group efficacy was 57.39% on D7 ($p < 0.05$), 92.79% on D14 and 100% on D28 and D35 ($p < 0.01$). The application of the spot on formulation moxidectin 2.5% and imidacloprid 10% was highly effective in the treatment of canine thelaziosis caused by *T. callipaeda*. Advocate[®] spot on can be recommended for the control of *T. callipaeda* infection, considering that this formulation is currently licensed in Europe for the treatment of a wide range of parasites affecting dogs.

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1. Introduction

Since the first diagnosis of eyeworm infection by *Thelazia callipaeda* (Spirurida, Thelaziidae) in dogs from northern Italy (Rossi and Bertaglia, 1989), this parasitosis has been increasingly reported in several European countries including Italy, Belgium, France, Greece, Portugal, Spain, and Switzerland (Diakou et al., 2015; Otranto et al.,

2015). In Europe, *T. callipaeda* has been documented as agent of ocular infection in domestic animals (dogs and cats) and wildlife (e.g., foxes, hares, rabbits) (Otranto et al., 2009). Recently the number of cases of thelaziosis in dogs, cats and foxes living in Eastern European countries has surged (Hodžić et al., 2014; Tasić-Otašević et al., 2016; Colella et al., 2016). Due to the former distribution in East Asian countries (e.g., China, Thailand, Japan and many others) this nematode was referred as the “oriental eye-worm” being recognised also as a causative agent of human infection (Shen et al., 2006). However, due to the large number of reports in European countries this common name of *T. callipaeda* is no longer appropri-

* Corresponding author.

E-mail address: domenico.otranto@uniba.it (D. Otranto).



Fig. 1. Eyeworm infection by *Thelazia callipaeda* in a dog.

ate. In Europe, cases of human thelaziosis have been reported in Italy (Otranto and Dutto, 2008), Spain (Fuentes et al., 2012), Croatia (Paradžik et al., 2016) and, Serbia (Tasić-Otašević et al., 2016) where autochthonous infections are well established in animal population. The increased number of eyeworm infections in both animals and humans is related to the presence of *Phortica variegata* (Diptera, Drosophilidae, Steganinae) that acts as the intermediate host of *T. callipaeda* in Europe (Otranto et al., 2006; Máca and Otranto, 2014) while feeding on lachrymal secretions of infected animals.

Both adult and larval stages of the eyeworm are responsible for symptoms ranging from mild (e.g., lacrimation, ocular discharge, epiphora) to severe (e.g., conjunctivitis, keratitis and, corneal opacity or ulcers), also related to the load of parasites inhabiting the orbital cavity (Shen et al., 2006). Nematodes (i.e. adult and larvae) may be removed mechanically by rinsing the conjunctival sac with saline solutions and adults may be collected with fine forceps or swabs (Shen et al., 2006).

Successful treatment of eyeworm infection included topic instillation of organophosphates (Rossi and Peruccio, 1989) or moxidectin 1% (Lia et al., 2004) which were effective against *T. callipaeda*. Nonetheless, topical administration is an off label use and may be irritant to the ocular tissues, therefore it is unacceptable in veterinary practice. Additionally, milbemycin oxime/praziquantel tablets (Milbemax[®], Novartis-Animal Health), at the minimal dose of 0.5 mg/kg milbemycin oxime, have shown to significantly reduce infection rate in naturally infected dogs and cats (Motta et al., 2012) and it is the only product so far registered for the treatment of canine thelaziosis in Europe. Meanwhile, imidacloprid 10% and moxidectin 2.5% spot-on formulation was shown to be effective for the treatment and prevention of dog thelaziosis (Bianciardi and Otranto, 2005; Lechat et al., 2015).

The aim of this study was to evaluate the clinical efficacy and safety of imidacloprid 10% and moxidectin 2.5% spot-on, in comparison to milbemycin oxime/praziquantel tablets as positive control, in the treatment of canine thelaziosis in naturally infected dogs.

2. Materials and methods

This study was conducted as a GCP negative and positive controlled, blinded and randomised field study in privately owned dogs living in a *T. callipaeda* endemic area of Basilicata region (southern Italy). Study animals were located in areas endemic for canine thelaziosis where the presence of the vector and of the disease in dogs was already reported (Otranto et al., 2003a). Forty-seven dogs (27 females and 20 males) aged 7 months to 13 years, of different breeds, in good health condition and with at least one live *T. callipaeda* adult in one eye were included in the study (Fig. 1), fol-

lowing collection of informed owner consent form. Details of study population are provided in Table 1.

At inclusion (Day (D) 0), animals were physically examined and both eyes were inspected, including a thorough examination underneath the third eyelid to detect and count live adult *T. callipaeda*. Clinical signs indicative of eyeworm infection, such as lacrimation, conjunctivitis, ocular discharge, keratitis and, ulcers, were recorded and classified as absent, mild, moderate or severe. Each dog was then weighed and assigned in accordance with a random treatment allocation plan to one of the treatment groups (G1: imidacloprid 10% and moxidectin 2.5% spot on, G2: Untreated control and G3: milbemycin oxime/praziquantel tablets). Both products were administered according to label instructions. In particular, Advocate[®] spot-on to provide a minimum dose of 2.5 mg moxidectin and 10 mg imidacloprid/kg and Milbemax[®] to provide a minimum dose of 0.5 mg/kg milbemycin oxime and 5 mg/kg praziquantel.

On D7, D14, D28, D35 dogs were physically examined and the infection level was assessed by examination of both eyes, including conjunctival pouch and third eyelid for live adult *T. callipaeda* count and clinical scores. On D7, animals in G3 received a second treatment with milbemycin oxime/praziquantel tablets according to product label. On D28, animals in G1 received a second treatment with imidacloprid 10% and moxidectin 2.5% spot-on. At study closure on D35, the presence of *T. callipaeda* larvae was assessed by microscopic examination of samples collected by conjunctival pouch flushing with 5 mL of saline solution (0.9%) and preserved in sterile tubes. Subsequently, *T. callipaeda* adults were removed. The tube was centrifuged for 5 min at 700g. The supernatant was aspirated and the sediment (1 mL solution) was analysed under an optical microscope (40×) for larval count. Nematode larvae and adults were morphological identified according to Skrjabin et al. (1971) and Otranto et al. (2003b).

From D0 to D35 all the dogs were observed daily by the owners to assess and record abnormalities of the general health and, in case of abnormal health conditions, the veterinarian was responsible to examine the dog and to record the results of the clinical examination.

2.1. Statistical analysis

Sample size was based on superiority of imidacloprid 10% and moxidectin 2.5% spot-on compared to the untreated control with respect to the percentage reduction of dogs harbouring adult *T. callipaeda* worms. A minimum sample size of 10 dogs per group was calculated based on an expected prevalence of non-infected dogs of 90.47% (Bianciardi and Otranto, 2005) in treated group and of 23.8% (Motta et al., 2012) in untreated control group, with 95% Confidence Level and 90% Power. In order to account for potential dropouts, the minimum number of animals to be included in the study was 15 in each group.

Dogs were allocated to study group following a random treatment allocation plan on the basis of an inclusion sequence, in blocks of three patients each. Each dog per block was randomly assigned to one of the three treatment groups. The primary variable for the efficacy evaluation was the number of dogs showing a complete elimination of adult eyeworms on D7, D14, D28, D35, by comparison of groups G1 and G2. Moreover, as secondary descriptive parameters, the efficacy of the treatment in the group G3 with respect to parasitological cure (therapeutic efficacy) and worm count/severity and/or presence of ocular clinical signs was calculated and compared with the untreated control group on D7, D14, D28, D35. Therapeutic efficacy (%) in the treatment of *T. callipaeda* infection was calculated for each time point using the following formula: Efficacy = [(% of animals positive in Untreated group – % of animals positive in Treated group) / % of animals positive in

Table 1
Data of the dogs enrolled in the study.

	Total	G1	G2	G3
Number of animals	47	16	15	16
Age [months]	38.6 (7–156)	36.7 (7–96)	37.5 (8–156)	41.6 (18–96)
Gender [%] Female/Male	57.4/42.6	56.3/43.8	60.0/40.0	56.3/43.8
Weight [kg]	14.9 (4.8–35.6)	14.7 (6.6–31)	14.0 (5.4–35.6)	15.8 (4.8–34.1)
Hair length [%]Long/Medium/Short	8.5/51.1/40.4	12.5/43.8/43.8	6.7/60.0/33.3	6.3/50.0/43.8

Table 2
Number and percentage of dogs positive for adult *Thelazia callipaeda* during the study.

Study day	0	7	14	28	35
G1	16 (100)	0	0	0	0
Efficacy [%]		100*	100*	100*	100*
G2	15 (100)	11 (73.33)	13 (87.67)	11 (73.33)	11 (73.33)
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G3	16 (100)	5 (31.25)	1 (6.25)	0	0
Efficacy [%]		57.39**	92.79*	100*	100*

*) $p < 0.01$ – (**) $p < 0.05$.

Untreated group] $\times 100$. Effectiveness was claimed if significant difference between groups G1 or G3 with the untreated control group (G2) was demonstrated by Fisher's Exact Test calculated on contingency tables for parasitological cure with 5% level of significance. In addition, the groups G1 and G3 were compared to each other by Fisher's Exact Test.

Worm count and ocular clinical signs reductions in the treated groups were compared at each time point (t) with the untreated control group as follows: % reduction $[t] = (C_{t0} - C_t) / C_{t0} \times 100$, where C_{t0} was the baseline count before treatment and C_t was the count at time t after treatment for each secondary variable under investigation. The significance of the worm count reduction in treated dogs was analysed by ANOVA, with standard statistical assumption.

Statistical analysis was planned and conducted in compliance with current guidelines (EMA, 2010). Statistical calculations and randomization were performed with: SPSS® statistical package for Windows, version 13.0, and nQuery + nTerim 3.0 (StatSols), Statistical Solutions® Ltd. 2014, Microsoft.

3. Results

The percentage of dogs with different levels of *T. callipaeda* infection and symptoms in each eye was homogenous among groups ($p < 0.05$). Number and percentages of dogs positive for *T. callipaeda* at each study day are reported in Table 2. Efficacy in group G1 (imidacloprid 10% and moxidectin 2.5% spot-on) was 100% at each day post treatment ($p < 0.01$). For the G3 group (milbemyacin oxime/praziquantel tablets) efficacy was 57.39% on D7 ($p < 0.05$), 92.79% on D14 and 100% on D28 and D35 ($p < 0.01$). G1 and G3 groups have significant different efficacy at D7 ($p < 0.05$) but not at the following time points. The total number and mean of live adult *T. callipaeda* worms counted in both eyes is presented in Table 3. The reduction of the number of worm counted in both eyes was 100% for the G1 group (imidacloprid 10% and moxidectin 2.5% spot-on) from D7 onwards and 90.91% on D7, 97.73% on D14 and 100% on D28 and D35 for the G3 group (milbemyacin oxime/praziquantel tablets). A natural mild reduction in the control group G2 was also observed (29.31%, 24.14%, 34.48% and 18.97% on D7, 14, D28 and D35 respectively). The reduction of the mean worm count for *T. callipaeda* from both eyes was significantly different ($p < 0.01$) for the G1 and the G3 group when compared to the control group at all post-treatment time points.

Ocular signs associated with *T. callipaeda* infection included lacrimation, conjunctivitis and discharge. None of the dogs showed

keratitis or ulcers except one dog in the G3 that showed both symptoms on D7. Due to the low occurrence of the different ocular signs, only lacrimation and conjunctivitis have been statistically analysed. No overall evidence of adverse effects of the treatments in the G1 (imidacloprid 10% and moxidectin 2.5% spot-on) and the G3 (milbemyacin oxime/praziquantel tablets) groups were demonstrated when compared to the control group and a significant difference was only observed between G2 and G3 for conjunctivitis affecting left eye on D28.

4. Discussion

The application of the spot on formulation moxidectin 2.5% and imidacloprid 10% (Advocate®) was highly effective in the treatment of canine thelaziosis. This formulation showed to be more effective in the treatment of thelaziosis compared to the administration of milbemyacin oxime/praziquantel tablets (Milbemax®). Indeed, no worms were detected following the first treatment, while dogs in the milbemyacin oxime/praziquantel tablets group still had worms counted on D7 and D14 (i.e. for one week after the second treatment). These data are in accordance with previous investigations where a single or two treatments with milbemyacin oxime/praziquantel tablets were effective within seven (72.7%) and 14 (90.9%) days after the administration of the product (Motta et al., 2012). A single application of imidacloprid 10% and moxidectin 2.5% spot-on was effective within five (90.47%) to nine (95.23%) days after administration (Bianciardi and Otranto, 2005) for the treatment of infected dogs. In this study, the spot on formulation moxidectin 2.5% and imidacloprid 10% resulted in the elimination of 100% of the eyeworms in infected animals within seven days from a single application, whereas two administrations of milbemyacin oxime/praziquantel tablets were required to completely cure *T. callipaeda* infection 21 days following the second administration. Moxidectin is absorbed through the skin, reaching maximum plasma concentrations approximately 4–9 days after treatment in dogs. Following absorption from the skin, moxidectin is distributed systemically and is slowly eliminated from the plasma as manifested by detectable moxidectin concentrations in plasma throughout the treatment interval of one month (EMA Advocate, 2016).

The monthly application of milbemyacin oxime (Ferroglio et al., 2008) as well as imidacloprid 10% and moxidectin 2.5% spot-on (Lechat et al., 2015) showed an efficacy in reducing eyeworm infection rate of 90% and 100%, respectively. Therefore, the application of imidacloprid 10% and moxidectin 2.5% spot-on could be success-

Table 3
Number (and mean) of adult *Thelazia callipaeda* adult live worms counted in both eyes.

Study day	0	7	14	28	35
G1	79 (4.94)	0	0	0	0
Reduction [%]		100*	100*	100*	100*
G2	58 (3.87)	41 (2.73)	44 (2.93)	38 (2.53)	47 (3.13)
Reduction [%]		29.31	24.14	34.48	18.97
G3	88 (5.50)	8 (0.50)	2 (0.13)	0	0
Reduction [%]		90.91*	97.73*	100*	100*

(*) $p < 0.01$.

fully used for both the treatment and prevention of thelaziosis in dogs. Considering that in endemic areas the prevalence of the infection may be up to 60%, such as in some municipalities of southern Italy (Otranto et al., 2003a), a continuous treatment of animals is central for the control of the infection, eventually reducing the risks for human health. Meanwhile, the prophylaxis against eyeworm infection in travelling dogs from a non endemic to an endemic area and *vice versa* is mandatory to limit the introduction of *T. callipaeda* in non endemic regions where the competent vector, *P. variegata*, is present (Otranto et al., 2006). The safety of the easy-to-apply of imidacloprid 10% and moxidectin 2.5% spot-on solution in the treatment of canine thelaziosis is inferred by the lack of any adverse effects and clinical signs compared to the control group.

5. Conclusion

Advocate® spot on can be recommended for the control of *T. callipaeda* infection, considering that this formulation is currently licensed in Europe for the treatment of a wide range of parasitic conditions of the dog (e.g., *Ctenocephalides felis*, *Sarcoptes scabiei* var. *canis*, *Demodex canis*, *Angiostrongylus vasorum*, *Toxocara canis* and *Uncinaria stenocephala*) and in the prevention of *Dirofilaria immitis* and *Dirofilaria repens*. Finally, since cases of animal and human thelaziosis are increasingly reported in Europe, the prevention and treatment of infected dogs is crucial for the control of this zoonotic infection.

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