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Research Article

EFFICACY COMPARISON OF AFOXOLANER/MILBEMYCIN OXIME WITH IVERMECTIN/PRAZIQUANTEL IN DOGS NATURALLY INFECTED WITH *TOXOCARA CANIS.*

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A B S T R A C T

The aim of the present study was to compare the efficacy of afoxolaner/milbemycin oxime with ivermectin/praziquantel in dogs naturally infected with *Toxocara canis*. *Toxocara canis* positive dogs (N = 200) were chosen by means of coproparasitoscopic analysis. Two study groups of 100 dogs each, which were treated with a single dose,one with 2.50–5.36 mg/kg of afoxolaner and 0.50–1.07 mg/kg of milbemycin oxime and another with ivermectin/praziquantel administered at doses of 0.2 mg/kg and 5 mg/kg. Samples were examined using the concentration-flotation technique, one on day 0 before treatment and at 7, 14 and 28 days post-treatment. Both treatments showed a reduction of dogs positive for *Toxocara canis*. Treatment with afoxolaner/milbemycin oxime decreased the parasite by 78% at 7 days (p = 0.01), 90% at 14 days (p = 0.02) and 96% at 28 days post-treatment (p = 0.0002) compared to 61% at 7 days, 77% at 14 days and 70% at 28 days post-treatment in dogs treated with ivermectin/praziquantel. The group treated with afoxolaner/milbemycin oxime showed a greater decrease in the number of dogs positive for *Toxocara canis* in comparison with the group that was administered ivermectin/praziquantel.

INTRODUCTION

The close relationship of people with their pets has formed a human-animal bond that provides benefits with respect to socialisation, mental health and even physical well-being (Paul *et al* 2010). Along with these benefits of pets for the human population, there are also potential health risks associated with the ownership of a pet as they harbour enteric nematodes (Overgaauw and van Knapen 2013). A particularly important example is represented by toxocariasis, a zoonosis caused by nematodes belonging to the *Toxocara* genus, which includes more than 30 species. In this genus, *Toxocara canis* causes the majority of problems (Nijsse *et al* 2016), generating clinical disorders such as vomiting, diarrhoea, anorexia and anaemia, especially in puppies and young dogs, affecting their development and increasing morbidity and mortality (Rehbein *et al* 2017).

T. canis has a complex life cycle that can involve paratenic hosts, including humans, particularly children (Zhu *et al* 2015), where *T. canis* larvae can invade various tissues causing visceral larva migrans, ocular larva migrans, meningoencephalitis and/or covert toxocariasis (Macpherson 2013).

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The presence of dogs in public areas, for example parks and recreational gardens, creates the ideal interface for infection due to widespread faecal contamination of these places (Fankhauser *et al* 2016).

Improving hygiene, promoting canine health care, including veterinary care, and, above all, using highly effective anthelmintics provide appropriate management practices for the control of these nematodes (Rojas *et al* 2017). Milbemycin oxime in its chewable formulation, which is easy to administer, has been used in the treatment and control of canine nematode infections since the beginning of the 1990s and has proven to be a highly effective and safe treatment alone and in combination with other antiparasitic agents for the treatment of *Toxocara* spp. (Hunter *et al* 2014; Rehbein *et al* 2017). The aim of the present investigation is to compare the use of afoxolaner in combination with milbemycin oxime versus ivermectin/praziquantel in the treatment of dogs naturally infected with *Toxocara* spp.

MATERIALS AND METHODS

The present study was carried out from August 2016 to March 2017 in the Parasitology Laboratory of the Veterinary Clinic of Companion Animals (CLIVAC) of the University Center UAEM Amecameca. The study was reviewed and endorsed by

the ethics committee of the Autonomous University of the State of Mexico. Registry number: CBA/AmUAEM/21/2016.

Selection of animals

We included 200 dogs, of which 109 were females and 91 males, with a positive diagnosis of *Toxocara canis* confirmed by coproparasitoscopic analysis of faecal samples collected directly from the rectum of the animals. The samples were analysed by the concentration-flotation technique. Inclusion criteria were dogs of any race and sex, less than 6 months old and with the prior consent of the owner by a letter of consent. Dogs that were dewormed 2 months before the study were excluded. Each of the animals was identified by a clinical leaf consisting of specific dog data, dose and anthelmintic drug with which it was treated.

Sample analysis

Examination of each faecal sample was performed as follows: day 0 initial sample for inclusion in the study and samples on days 7, 14 and 28 post-treatment. The samples were analysed by the coproparsitoscopic technique of concentration-flotation centrifugation using a solution of zinc sulphate with a specific density of 1.18 g/mL. As a modification of Coelho *et al*., the faeces sample was taken directly from the rectum and centrifuged 200g for 1 minute three times. Once processed, the sample was observed under a microscope with 4x, 10x, 20x and 40x objectives for the identification of parasitic forms. *T. canis* eggs were identified according to the morphology of the AAVP (American Association of Veterinary Parasitologists) guide (Zajac and Conboy 2012). The result of the analysis was reported as the presence or absence of *Toxocara canis*.

Treatment

The dogs were randomly divided into two groups: 100 canines were treated with 2.50–5.36 mg/kg of afoxolaner and 0.50–1.07 mg/kg of milbemycin oxime (Nexgard Spectra®), and the other group of 100 dogs was treated with 0.2 mg/kg of ivermectin combined with 5 mg/kg of praziquantel tablets (Endovet® Ces). On the day of positive diagnosis of *Toxocara* spp., a single dose of treatment was administered.

Statistic analysis

The data collected during the study were analysed. First, the distribution of the data was determined. Since they did not have a normal distribution, they were analysed using nonparametric statistics. Fisher's exact two-tailed test was applied with an alpha of 0.05 using JMP 8.0 software (Wittington House Marlow, Buckinghamshire).

RESULTS

At the beginning of the study, 100% of the dogs included tested positive for *Toxocara canis*, so no comparison was made to this measurement between groups and only post-treatment values were contrasted. Results of the comparison between treatments were observed throughout the study from day 0 pre-treatment to 7 days post-treatment, where there was a significant difference ($p = 0.01$) between the individuals treated with afoxolaner/milbemycin oxime, with a 78% reduction of dogs positive for *Toxocara canis*, against ivermectin/praziquantel that showed a 61% reduction of *Toxocara canis* at 7 days post-treatment. At 14 days post-treatment, the analysis yielded the following result: afoxolaner/milbemycin oxime showed an efficacy of 90% and ivermectin/praziquantel 77%, presenting a significant difference ($p = 0.02$). At 28 days post-treatment, there was also a significant difference ($p = 0.0002$) between the group treated with afoxolaner/milbemycin oxime, which showed a 90% reduction in positive animals, and the group treated with ivermectin/praziquantel showed a 70% reduction in positive animals, presenting a higher value compared to the previous measurement of 77% at 14 days. Overall, a constant decrease in positive animals was observed in the group treated with afoxolaner/milbemycin oxime throughout the study (Table 1).
The comparison between males and females was made by treatment. Those treated with afoxolaner/milbemycin oxime at 7, 14 and 28 days post-treatment did not present a significant difference (Table 2). Similarly, there was no significant difference between males and females treated with ivermectin/praziquantel (Table 3).

The comparison of females between treatments at 7 and 14 days showed a trend ($p = 0.07$) of a greater decrease of positive dogs with the administration of afoxolaner/milbemycin oxime. At 28 days, there was a significant difference ($p = 0.003$) in the decrease of positive dogs between the groups of 94.6% treated with afoxolaner/milbemycin of oxime and 73.6% treated with ivermectin/praziquantel (Table 4). In the comparison of the males between treatments, no significant difference was found during the study (Table 5).

DISCUSSION

The results of the present investigation show that treatment with afoxolaner/milbemycin oxime exhibits a reduction of dogs positive for eggs of *Toxocara canis* of 78% at 7 days, 90% at 14 days and 96% at 28 days post-treatment (Table 1), which corresponds to similar results in a study conducted by Fankhauser *et al*., in which they demonstrate efficacy in the combination of afoxolaner plus milbemycin oxime of 98% against *T. canis* with a minimum dose of 2.5 mg + 0.5 mg per kg of body weight, performing evaluations at 7 days post-treatment by obtaining samples from the digestive tract of infected animals. In another similar study, Rehbein *et al*., administered the same minimum therapeutic dose, which revealed an efficacy of 99.4% against *T. canis* at 7 or 8 days post-treatment using McMaster techniques to detect nematode eggs. The results obtained from the present study are similar to the aforementioned, but it should be noted that they reached ranges above 96% at 7 or 8 days post-treatment; in this investigation, it was reached at 28 days post-treatment.

Bowman et al. reported an efficacy of 96.15% at 24 days post-treatment using milbemycin oxime with spinosad in 32 dogs 3 to 4 months of age inoculated with 250 *T. canis* eggs without reporting a difference in efficacy by gender, as opposed to this study in which the treatment of afoxolaner/milbemycin oxime worked better in the females at 28 days post-treatment, showing a significant reduction ($p = 0.003$) of positives for *Toxocara canis* compared with females treated with ivermectin/praziquantel.

In another study, Rehbein *et al*., evaluated the safety and efficacy of afoxolaner plus milbemycin oxime chewable tablets compared to milbemycin oxime plus praziquantel against gastrointestinal nematode infections, including *T. canis*, in dogs. Dogs treated with afoxolaner plus milbemycin oxime showed a better result in terms of reduction of the faecal

egg count compared to dogs treated with milbemycin oxime plus praziquantel, which was 99.7% and 99.5%, respectively.

The macrocyclic lactones have excellent anthelmintic activity (Wright and Elsheikha 2018) and are safe for use in pregnant females and in puppies 8 weeks and older not showing anomalies in their health (Drag *et al* 2017). Studies of safety directed at animals with multiple doses and variables of milbemycin oxime indicate that greater exposure has no implications in adverse clinical reactions (Holmstrom *et al* 2012).The results of this study show that tablets with a combination of afoxolaner and milbemycin oxime administered orally only once to dogs naturally infected by *T. canis* are safe and effective, becoming a viable option in the clinical and preventive management of this nematode when complications were not observed due to its administration.

Table 1 Comparison between number of dogs positive and negative for *Toxocara canis* treated with a dose of afoxolaner/milbemycin oxime or ivermectin/praziquantel with measurements at 7, 14 and 28 days post-treatment.

Day post-treatment	Positives (%)	Negatives (%)	Total	P[a]
Day 7				
AFO /MO	22 (22)	78 (78)	100	0.01[b]
I/P	39 (39)	61(61)	100	
Day 14				
AFO /MO	10 (10)	90 (90)	100	0.02[b]
I/P	23 (23)	77 (77)	100	
Day 28				
AFO /MO	4 (4)	96 (96)	100	0.0002[b]
I/P	30 (30)	70 (70)	100	

[a]Fisher's exact two-tailed test ($p < 0.05$)
[b]Significantdifference
AFO /MO = afoxolaner/milbemycinoxime, I/P = Ivermectin/Praziquantel

Table 2 Comparison between sexes treated with a dose of afoxolaner/milbemycin oxime with measurements at 7, 14 and 28 days post-treatment.

Day post-treatment	Positives (%)	Negatives (%)	Total	P[a]
Day 7				
Females	10 (17.9)	46 (82.1)	56	0.33
Males	12 (27.3)	32 (72.7)	44	
Day 14				
Females	6 (10.7)	50 (89.3)	56	0.72
Males	4 (9.1)	40 (90.9)	44	
Day 28				
Females	3 (5.4)	53 (94.6)	56	0.62
Males	1 (2.3)	43 (97.7)	44	

[a]Fisher's exact two-tailed test ($p < 0.05$)

Table 3 Comparison between sexes treated with a dose of ivermectin/praziquantel with measurements at 7, 14 and 28 days post-treatment

Day post-treatment	Positives (%)	Negatives (%)	Total	P[a]
Day 7				
Females	18 (34.0)	35 (66.0)	53	0.30
Males	21 (44.7)	26 (55.3)	47	
Day 14				
Females	13 (24.5)	40 (75.5)	53	0.81
Males	10 (21.3)	37 (78.7)	47	
Day 28				
Females	14 (26.4)	39 (73.6)	53	0.51
Males	16 (34.0)	31 (66.0)	47	

[a]Fisher's exact two-tailed test ($p < 0.05$)

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