Efficacy of Afoxolaner Plus Milbemycin Oxime in the Treatment of Canine Demodicosis

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ABSTRACT

Demodicosis is a canine dermatosis produced by the *Demodex* sp. mite; the localized form can become generalized and have bacterial complications, making it difficult to effectively treat. The objective was to evaluate the efficacy of the use of afoxolaner plus milbemycin oxime in the treatment of generalized canine demodicosis. Sixty-eight dogs diagnosed with generalized demodicosis were included. From each dog, three skin scrapings and hair samples were obtained. Dogs were treated with an oral dose of 2.50–5.36 mg/kg of afoxolaner in combination with 0.50–1.07 mg/kg of milbemycin oxime. Evaluations were made on days 7, 14, and 28 post-treatment through a lesion visualization and positive samples to mites.

After a single oral dose of afoxolaner with milbemycin oxime, the number of positive animals according to skin scrapings was reduced from 100% on day 1 to 17.6% on day 28 ($P < 0.05$). The resolution of the lesions after treatment was variable, with a significant decrease for the first 7 days. This parameter continued to decrease until the end of the study on day 28, when a decrease of 94.68% was observed. An association between the presence of *Demodex* sp. and the area of the head was observed (Chi-square = 14.65, $P = 0.0001$). Oral administration of a single dose of 2.50–5.36 mg/kg of afoxolaner in combination with 0.50–1.07 mg/kg of milbemycin oxime is effective in the treatment of generalised demodicosis in dogs, with a decrease in lesions in the first days of treatment.

INTRODUCTION

Demodex mites are considered to be part of the cutaneous microbiota of dogs. It is believed that all dogs have a small population of *Demodex* spp. mites in their skin,[1] but the manifestation of the disease is limited to some animals. Under normal conditions, they seem to live as commensals, feeding on the tallow of their hosts, thus acting as a pathogenic opportunists. They are transmitted from the bitch to her puppies during the first days of life[2] and are commonly found in the pilosebaceous follicles.[3]

Demodicosis is the result of an excessive proliferation of the *Demodex* spp. mite. One of the causes of this process is concurrent immunosuppressive conditions (specific hereditary immunodeficiency of *Demodex* spp. or acquired immunosuppression). Studies have identified the induction of demodicosis by the suppression of the immune response and observed the development of the disease in strains of immunodeficient rats, in addition to numerous clinical occurrences in immunosuppressed humans and animals. These studies have shown that immunosuppression is directly associated with the development of the disease, which is frequently found in dogs and rarely found in cats, resulting in skin lesions consistent with demodicosis.[4,5] It typically occurs in young dogs (between 3 months and 1 year), although there are reports in dogs from two weeks of age[6] and in dogs older than one year, these cases tend to be occasional[3] or secondary to another underlying pathology.[7]

Canine demodicosis is a common, non-contagious parasitic dermatosis[8] that can be divided into two clinical manifestations: localized and generalized. Localized demodicosis appears as small alopecic and erythematous areas, which can regress spontaneously without the administration of treatment. Generalized demodicosis is more variable and is characterized by five or more affected areas or lesions that cover an entire region of the body, and/or pododemodicosis that involves two or more legs; the affected areas are erythematous, with comedones, alopecia, follicular pustules or papules and scales.[9] It can be fatal in severe chronic cases with secondary bacterial infection[10] and progression of the disease is associated with lymphadenopathy, lethargy, and fever.[2]

Diagnosis of demodicosis is usually based on clinical signs, and is confirmed by the presence of mites on deep skin scrapings,[2] allowing for identification at different stages of mite development.[3] The management of canine demodicosis includes multimodal treatment,[8] in addition to an effective acaricide therapy.

The treatment of internal parasitism is suggested to maximize the potential of a successful treatment.[2] The currently available therapeutic options include:

- Amitraz[2,4]
- Ivermectina[11]
- Imidacloprid-moxidectin[12,13] or
- Doramectin[14]

Regardless of the antiparasitic drug chosen, several months of treatment, with lasting from three months or more, are often required to achieve a clinical resolution.[15] For all therapy options, the benefits and risks associated with the treatment must be considered. For example, poor safety or toxicity from the amitraz and and environmental

contamination problems are particularly associated with avermectins.[16]

Isoxazolines have opened a new avenue in the therapeutic treatment of demodicosis. Afoxolaner is an isoxazoline that has both insecticidal and acaricidal activity, acting on GABA/glutamate receptors to inhibit chloride ion channels, producing irreversible neuronal hyperexcitability and the death of arthropods.[17,18] Afoxolaner has been evaluated in dogs with generalised demodicosis using monthly oral administration. It has been shown to effectively reduce live mites compared to control groups, as assessed through skin scrapings and clinical improvement in dermatological lesions, without any adverse effects,[15,19] thus achieving a high level of efficacy for the control of this dermatosis. Currently, there is an oral chewable tablet formulation[15] that combines the insecticidal-acaricidal compound of afoxolaner with the nematocidal compound of milbemycin oxime (NexGard Spectra®, Boehringer Ingelheim)[20,21] to provide treatment and control of endo- and ectoparasites in dogs.[22]

In addition, milbemycin oxime has also been shown to be useful for the treatment of generalized canine demodicosis at doses of 0.5 to 2 mg/kg orally once a day.4 Due to its ease of use, safety and efficacy, it can be used in all dog breeds, including Collies and Shetland Sheepdogs.[23] However, there are no reports on the use of this isoxazoline combined with milbemycin oxime for the treatment of dogs with demodicosis. Therefore, the aim of this study was to evaluate the efficacy of the use of afoxolaner plus milbemycin oxime in the treatment of generalised canine demodicosis after 28 days of treatment.

MATERIALS AND METHODS

This study protocol was approved by the Ethics Committee of the Amecameca University Center of the Autonomous University of the State of Mexico (UAEM). Sixty-eight dogs from the State of Mexico, Mexico City, and Guadalajara, Mexico, were included, with the prior consent of the owner, by means of a permission letter

ANIMALS

Inclusion criteria were: animals with a minimum age of 8 weeks and weighing more than 2 kg with parasitic evidence of at least four live *Demodex* spp. mites (immature or adults), confirmed by positive deep skin scrapings and observation of hair samples under a microscope. These are in addition to clinical evidence of demodicosis such as:

- Alopecia
- Erythema
- Comedones
- Papules
- Pustules
- Scales, or
- Scabs.

Generalized demodicosis was defined as a complete body region or five or more localized lesions (diameter > 2.5 cm) or pododemodicosis that involved two or more limbs.[2] Exclusion criteria were: pregnant or lactating bitches, animals undergoing immunosuppressive therapy, or having been treated with an ectoparasitic with residual efficacy against *Demodex* spp.

Treatment Protocol

Day 1 (time of positive skin scrapings), dogs were treated orally with 2.50–5.36 mg/kg of afoxolaner and 0.50–1.07 mg/kg of milbemycin oxime (NexGard Spectra®). The tablets were administered directly into the oral cavity 10 minutes after eating. Skin scrapings were repeated on days 7, 14, and 28. Data were recorded as the presence or absence of live mites and a dermatological lesion score.

Diagnostic Tests

Three surface scrapings (5 x 5 cm) and hair samples, and four scrapes of deep skin (1 x 1 cm) were taken from the most affected areas. During the procedure, pressure was applied to the area to be sampled to bring the mites to the surface. Areas with primary lesions, such as papules and follicular pustules, were chosen. Sampling in ulcerated areas was avoided. A scalpel blade was used to collect

Table 1. *Comparison of the decrease in the presence of Demodex canis (as assessed by skin scapings) and number of lesions, after treatment with afoxolaner plus milbemycin oxime*

	Day 1	Day 7	Day 14	Day 28	CV	MEE
Positive samples (%)	68 (100)[a]	37 (54.4)[b]	13 (19.1)[c]	12 (17.6)[c]	104.70	0.03
Lesions	177.37[a]	74.10[b]	33.43[bc]	9.47[c]	158.16	7.05

[abc] *Means with different letters within a row represent a significant difference, P < 0.05*

the samples. In some cases, hair was pulled. The scraped material was transferred to a slide, mixed with mineral oil and examined microscopically using a Leica DFC300 FX® microscope. Adults, larvae, nymphs, and eggs were evaluated microscopically at 40x or 100x magnification. On all the sample dates, the previously affected zones were sampled. Dogs were considered cured when the scrapings were negative. During the treatment period, each dog remained in standard conditions at home, consuming their normal diets.

Statistical Analysis

Due to the fact that the obtained data did not follow a normal distribution, nonparametric statistics tests (Tukey's range test, Pearson correlation coefficient, Fisher's exact test, Chi-square test) were applied.

RESULTS

Of the 68 individuals who participated in the study, 33 were females and 35 were males, with a mean age of 10.5 (± 5) months; 18 dogs were more than 12 months old and 50 were less than 11 months old. In terms of breed, 16 were mixed dogs and 52 were purebred dogs. These dogs included:

- 7 Pit Bulls
- 4 Labrador Retrievers
- 4 Cocker Spaniels
- 4 Boxers
- 3 English Bulldogs
- 3 German Shepherds
- 3 Maltese
- 3 Pugs
- 2 Bull Terriers
- 2 Poodles
- 2 Rottweilers
- 2 Schnauzers
- 2 Dobermans
- 1 French Bulldog
- 1 Xoloitzcuintle
- 1 Dutch Shepherd
- 1 Chow Chow
- 1 Chihuahua
- 1 Border Collie
- 1 Argentine Dogo
- 1 Golden Retriever
- 1 Shar Pei
- 1 Old English Sheepdog, and
- 1 Belgian Shepherd Malinois.

There was a decrease in the number of animals positive for *Demodex* sp. by visualisation of scrapings and in number of lesions, with a positive correlation (PCC = 0.44181, P < 0.0001) between these variables. According to the values obtained for the presence of *Demodex* sp. in scrapings (Table 1), a significant decrease (P < 0.05) was observed in the number of positive animals from day 1 (100%) to day 7 (54.4%), and day 14 (19.1%). There were no significant differences in the number of mites between days 14 and 28 (19.1% and 17.6%, respectively).

In the comparison of lesions per week (Table 1), significant differences were found (P < 0.05) between day 1 (177.37) and day 7 (74.10). However, there were no significant differences between days 7, 14, and 28 (74.10, 33.43 and 9.47, respectively). As for gender and age (Table 2), no significant differences were found, but there was a progressive decrease until day 28. For the diagnosis of *Demodex* sp. samples taken from different areas of the body, there was an association between the presence of *Demodex* sp. and the area of the head (Chi-square = 14.65, P = 0.0001), but this association did

Table 2. *Demodex canis presence comparison per week, according to gender and age, after treatment with afoxolaner plus milbemycin oxime*

	Day 1 Pos./Neg.	Day 7 Pos./Neg.	Day 14 Pos./Neg.	Day 28 Pos./Neg.
Male	35/0	20/15	7/28	5/30
Female	33/0	18/15	7/26	7/26
***P value**	1	0.67	0.66	0.33
Puppy	50/0	27/23	10/40	10/40
Adult	18/0	10/8	3/15	2/16
***P value**	1	0.64	0.73	0.32

Pos. – positive; Neg. – negative
Puppy.- <1; Adult.- >1
** Fisher's exact test, P < 0.05*

not exist in the anterior and posterior limbs, nor in the back (Table 3).

DISCUSSION

The results of the present study demonstrate the efficiency and safety of 2.50–5.36 mg/kg of afoxolaner with 0.50–1.07 mg/kg of milbemycin oxime in dogs with generalised demodicosis. Injectable formulations of ivermectin for the control of generalized demodicosis in dogs have also been reported,[2] although with difficulties in the administration of doses and resistance to ivermectina.[11] In addition, there was sensitivity to macrocyclic lactones in some breeds and problems of environmental contamination.[2]

In the present study, after a single oral dose of afoxolaner with milbemycin oxime, there was a significant decrease in the number of mites from 82.4% to day 28, demonstrating that the combination of afoxolaner with milbemycin oxime has rapid acaricidal activity. The rapid decrease in the number of mites after the administration of afoxolaner with milbemycin oxime was also accompanied by a marked improvement in the index of the lesions.

In previous studies, Beugnet et al.[15] reported a scab index of 50% and a scale index of 12.5% in dogs treated with afoxolaner at day 28, with a reduction to 25% and 0%, respectively, at day 56. This was a superior treatment compared to dogs that received a formulation of moxidectin/imidacloprid, who demonstrated 87.5% scabs and 62.5% scales and 50% scabs and 12.5% scales at days 28 and 56, respectively. These data are similar to those reported with sarolaner, where a decrease in the rate of crusts, comedones, erythema, papules and pustules was observed on day 14 and 30, in addition to a decrease in the affected body area (21% at day 30 and 9% at day 60).[24] In the same way, Bezerra et al.[25] reported the use of fluralaner in generalised juvenile demodicosis, which led to a clinical improvement at day 10 after treatment and a complete resolution of the lesions in the fourth week. In the present study, a decrease in the index of the lesions

Table 3. *Association between body area and presence of Demodex canis, after treatment with afoxolaner plus milbemycin oxime*

Body area	Presence	Absence	Chi-squared	*P value
Head	58	10	14.65	0.0001
Anterior limbs	40	28	----	-----
Posterior limbs	34	34	-----	-----
Back	38	30	-----	-----

**P < 0.05*

was observed over the course of the treatment, reaching a score of 9.47 at day 28.

The results of the 68 individuals who participated in the study demonstrate that afoxolaner with milbemycin oxime is an option that is tolerated and effective for the treatment of generalised demodicosis in dogs, with no apparent adverse reactions by sex, race or age[20,26] for 28 days.

Treatment with an oral formulation of afoxolaner plus milbemycin oxime (NexGard Spectra®) results in a rapid decrease in the number of mites and marked clinical improvement in dogs with generalized demodicosis in the first days after treatment, providing a new treatment that combines safety, efficiency, and ease of use.

Declarations

Ethics Approval and Consent to Participate

This study protocol was approved by the Ethics Committee of the Amecameca University Center of the Autonomous University of the State of Mexico (UAEM).

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

All authors had a substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content and final approval of the version to be published.

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