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Efficacy of sarolaner, a novel oral isoxazoline, against two common mite infestations in dogs: *Demodex* spp. and *Otodectes cynotis*

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ABSTRACT

The efficacy of sarolaner (Simparica™, Zoetis) was evaluated against *Demodex* spp. in dogs with generalized demodicosis and against *Otodectes cynotis* (otodectic mange) in dogs with induced infestations.

In the first study, 16 dogs with clinical signs of generalized demodicosis and positive for *Demodex* spp. mites were randomly assigned to treatment with either sarolaner (2 mg/kg) orally on Days 0, 30 and 60, or topical imidacloprid (10 mg/kg) plus moxidectin (2.5 mg/kg) solution every 7 days from Day 0 to Day 81. For sarolaner-treated dogs, pretreatment mite counts were reduced by 97.1% at 14 days and 99.8% by 29 days after the first dose, with no live mites detected thereafter. Weekly imidacloprid plus moxidectin resulted in 84.4 and 95.6% reduction at these two time points, respectively, with no mites detected from Day 74 on. All dogs in both groups showed marked improvement in the clinical signs of demodicosis.

In the second study, 32 dogs with induced infestations of *O. cynotis* were randomly assigned (eight per group) to oral sarolaner (2 mg/kg) as a single treatment on Day 0 or as a two dose regime (Days 0 and 30), or a placebo group for each of the dose regimes. Sarolaner administered at 2 mg/kg as a single oral dose resulted in a 98.2% reduction at Day 30 and two doses of sarolaner, administered one month apart, resulted in a 99.5% reduction in ear mites at Day 60 compared to placebo controls. There were no treatment related adverse events in either study.

In these studies, sarolaner at an oral dose of 2 mg/kg was highly effective in reducing the live mite counts associated with a natural infestation of *Demodex* spp. and an induced infestation of *O. cynotis*. In addition, the *Demodex*-infested dogs showed a marked improvement in the clinical signs of generalized demodicosis.

1. Introduction

Demodicosis is an inflammatory parasitic disease of dogs characterized by the presence of larger than normal numbers of *Demodex* mites. Traditionally demodicosis was thought to be a disease caused by unchecked replication of *Demodex canis* mite though more recently *Demodex injai* and *Demodex cornei* (Forsythe et al., 2009; Sastre et al., 2012; Desch and Hillier, 2003) have also been identified in dogs with demodicosis. *Demodex* spp. mites are considered a normal resident of the dog's skin with transmission occurring from the dam to nursing neonates by direct contact during the first two or three days of life. Four stages may be demonstrated in skin scrapings; eggs, larvae, nymphs and adults (Muller and Kirk, 2013). Two types of demodicosis are recognized, localized and generalized, though there is no uniformly accepted standard as to how many localized lesions are needed before the disease is characterized as generalized. Diagnosis is made based on clinical signs and deep skin scrapings which confirm the presence of the mites.

Generalized demodicosis is a severe condition that is difficult to control with currently approved therapies. Saturation applications of amitraz solutions can be used for the treatment of generalized demodicosis, although the application process is cumbersome as it requires dipping or wetting the dog's entire body and/or sponging the product onto the dog for three to six treatments repeated every 14 days. Higher doses may increase efficacy but tend to be associated with increased adverse reactions (Mueller, 2004). Frequently reported side effects include a temporary sedative effect for 12–24 h, especially after the first treatment, and some dogs

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become pruritic. A number of macrocyclic lactones used at off-label dosages and regimes have been shown to provide varying levels of effectiveness against *Demodex* spp. mites, though often the high doses required can result in adverse reactions (Garfield and Reedy, 1992; Johnstone, 2002; Schnabl et al., 2010). High doses of oral ivermectin administered daily provided effective control of demodicosis over four month treatment intervals (Paterson et al., 2009, 2014) but signs of toxicity are occasionally seen in dogs on thes high dose regimes (Bissonnette et al., 2009). One macrocyclic lactone, moxidectin, in combination with imidacloprid is effective for the treatment of demodicosis when administered topically at monthly or in severe cases at weekly intervals (Heine et al., 2005; Fourie et al., 2009) and is approved with this indication in Europe. However, other clinical studies have reported lower efficacy with this product especially in dogs with moderate to severe clinical signs of demodicosis (Mueller et al., 2009) and confirmed that efficacy increased with frequency of application and treatment at weekly intervals was most effective (Paterson et al., 2009).

Otoacariasis or otodectic mange is caused by *Otodectes cynotis*, an obligate parasite which inhabits the vertical and horizontal ear canals of dogs and cats. Dogs infested with *O. cynotis* most commonly develop otitis externa characterized by vertical and horizontal canal erythema and a dark brown, ceruminous otic exudate. In addition to otitis externa, infestations of the head, neck, tail head and trunk can occur when the mites "escape" the ear canals. Puppies appear to be most susceptible and zoonotic infections have also been reported (Curtis, 2004; Muller and Kirk, 2013). Diagnosis of *O. cynotis* infestation is made by detection of mites within the ceruminous debris from an infected ear canal or by visualization of mites on otoscopic examination.

A number of liquid aural preparations containing antifungals, antibiotics, steroids and/or parasiticides are licensed for the treatment of *O. cynotis* infestations in dogs, but these have a limited residual action and require regular re-application for several days. Extra-label aural administration of products containing pyrethrins and ivermectin (Muller and Kirk, 2013), and fipronil (Bourdeau and Lecanu, 1999 Vincenti and Genchi, 1997) have also been reported to be efficacious on some occasions. Topical spot-on formulations including the macrocyclic lactones, selamectin and moxidectin, administered monthly are effective for the treatment and control of *O. cynotis* infestations in dogs (Krieger et al., 2005; Six et al., 2000; Arther et al., 2015). Moxidectin administered orally, extra-label, at a dose of 0.2 mg/kg twice at 10 day intervals and has also been shown to be effective (Muller and Kirk, 2013). Systemic products with residual action offer an attractive therapeutic option for veterinarians and owners.

Sarolaner (Simparica™, Zoetis) is a novel isoxazoline with potent activity against ticks and fleas (McTier et al., 2016), and sarcoptic mange mites (Becskei et al., 2016) following oral administration. Two exploratory studies were conducted in dogs to evaluate the efficacy and safety of sarolaner against natural infestations of *Demodex* spp. and induced aural infestations of *O. cynotis* at the minimum dose of 2 mg/kg proposed for monthly administration for the treatment and control of fleas and ticks.

2. Materials and methods

Two laboratory studies were conducted in the Republic of South Africa in compliance with Good Clinical Practice (EMEA, 2000). Study protocols were reviewed and approved by ClinVet and Zoetis Institutional Animal Care and Use Committees. Masking of both studies was assured through the separation of functions. All personnel conducting observations or animal care, or performing skin scrapings and or counts were masked to treatment allocation.

2.1. Animals

In both studies, dogs were individually housed in enclosures that allowed for auditory and visual contact without physical contact and conformed to accepted animal welfare guidelines. Each dog was individually identified by an alphanumeric microchip implant. The dogs had not been treated with an ectoparasiticide for at least 90 days and were in good health at treatment. Dogs were fed an appropriate maintenance ration of a commercial dry canine feed for the duration of the study. Water was available *ad lib*.

The *Demodex* study included 16 (6 male and 10 female), locally sourced, adult mongrel dogs ≥6 months of age and weighing from 6.2 to 23.1 kg. Dogs were enrolled in the study as suitable animals with natural *Demodex* infestations were identified. Twelve dogs were included in an initial enrolment and a further four dogs were added approximately three months later. All enrolled dogs exhibited clinical signs of generalized demodicosis; skin lesions such as alopecia, erythema, comedones, papules, pustules, casts, scales or crusts involving an entire body region or five or more localized lesions containing areas (each with a diameter >2.5 cm), or pododemodicosis involving two or more feet, and a total of at least four live *Demodex* mites in deep skin scrapings (Muller and Kirk, 2013; Mueller et al., 2012). In addition, they had not received a short acting corticosteroid within one week or a long acting corticosteroid within four weeks of Day 0.

Thirty-two (17 male and 15 female) adult, purpose bred Beagles and mixed breed dogs, 1–7 years of age and weighing from 8.0 to 24.8 kg were used in the *Otodectes* study. *O. cynotis* infestations had been previously induced in these dogs by harvesting mites by lavage from donor dogs with patent natural infestations and transferring approximately 100 mites into each of the ears of the recipient dogs. On Day −4, the presence of live mites was confirmed by otoscopic examination.

2.2. Experimental design and methods

General methods: Day 0 was defined as the day an animal received its first treatment. Dogs were acclimated to the study conditions for at least 14 days prior to treatment. A veterinarian performed a physical examination on each dog to determine health and suitability prior to inclusion in the study, and general health observations were made at least once daily throughout the study. Oral dosing was conducted in the fed state. Food was withheld the evening prior to the morning of treatment, and dogs were offered their standard ration one hour prior to dosing. Tablets were orally administered by pilling to ensure accurate and complete dosing. Each dog was observed for several minutes, and then at approximately two hours after dosing for evidence that the dose was completely consumed. At 1, 3, 6 and 24 h post-dosing, all dogs were observed for any signs of abnormal health. To avoid cross-infestation or contamination of dogs during mite counts, study personnel changed protective clothing between dogs in each treatment group, utilized separate leashes, gloves and equipment with each dog, and cleaned the surface of the examination Table used for scraping and/or mite counting.

In the *Demodex* study, demodicosis was assessed by mite counts and evaluation of clinical signs. Mite infestations were evaluated using deep skin scrapings taken from five primary sites showing the most severe clinical evidence of mite infestation based on visual examination. The same five primary sites were scraped on all days on which mites were counted. Scraped material was transferred to a slide, mixed with mineral oil and examined under a microscope using 40× or 100× magnification to count adult and immature mites. The clinical signs of demodicosis were assessed as the percent of the body surface affected by skin lesions followed by the assignment of a clinical score to each of four parameters: (1)

Table 1
Geometric mean live *Demodex* mite counts, range, percent efficacy relative to pretreatment and the proportion of dogs with no live mites for dogs treated with three monthly oral doses of sarolaner or receiving weekly topical applications of imidacloprid plus moxidectin.

		Study day						
		−4	14	29	44	59	74	91
Sarolaner (n = 8)	Mean count	260.5	7.5[*,**]	0.4[*,**]	0.0[*]	0.0[*]	0.0[*]	0.0[*]
	Range	4–1275	0–315	0–8	0–0	0–0	0–0	0–0
	Efficacy (%)	–	97.1	99.8	100	100	100	100
	Mite free (%)	0	37.5	75	100	100	100	100
Imidacloprid plus moxidectin (n = 8)	Mean count	243.2	37.8[*]	10.8[*]	0.7[*]	0.1[*]	0.0[*]	0.0[*]
	Range	17–2820	0–1733	0–383	0–9	0–1	0–0	0–0
	Efficacy (%)	–	84.4	95.6	99.7	>99.9	100	100
	Mite free (%)	0	37.5	37.5	62.5	87.5	100	100

[*] Post-treatment geometric mean live mite counts significantly lower than pre-treatment ($P < 0.0001$).
[*,**] Geometric mean live mite counts for sarolaner significantly lower than imidacloprid plus moxidectin ($P < 0.0331$).

comedones, pustules and papules, (2) casts, crusts and scales, (3) alopecia and (4) erythema. Following the Day −4 assessments, dogs were ranked in blocks of two by descending mite count and randomly allocated to treatment with either sarolaner (Simparica™, Zoetis) or the positive control, topical imidacloprid plus moxidectin (Advocate®/Advantage® Multi Spot-on solution for Dogs, 100 mg imidacloprid + 25 mg moxidectin/mL, Bayer). Dogs in the sarolaner group were dosed orally with a single tablet that was shaved or sanded to provide the target dose of 2 mg/kg of sarolaner on Days 0, 30 and 60. Positive control dogs were dosed topically once weekly from Day 0 to Day 81 with the appropriate band dose providing ≥10 mg imidacloprid + ≥2.5 mg moxidectin/kg, per European label directions for dogs with severe generalized demodicosis. Doses were calculated based on the most recent body weights determined on Days −3, 29 and 59. All dogs were examined for the clinical signs of demodicosis and skin scrapings/mite counts were performed on Days 14, 29, 44, 59, 74 and 91.

In the *Otodectes* study, dogs were weighed and examined otoscopically on Day −4, and 32 animals positive for live mites were selected for inclusion in the study. Sixteen animals were allocated to a single treatment on Day 0, the other 16 to a two dose treatment regime (Days 0 and 30). Within each dosing regimen, dogs were assigned to treatment with either placebo or sarolaner oral tablet (eight per group) and to individual pens using a randomized complete block design. Dogs in the sarolaner-treated groups were dosed orally with single tablets shaved and/or sanded to provide the target dose of 2 mg/kg. Control dogs received single placebo tablets. Day 0 dose was calculated using the Day −4 body weight and dogs receiving two doses were also weighed on Day 28 for calculation of the Day 30 dose. Dogs in the single dose groups were examined otoscopically for the presence of live mites on Day 14 and total ear mite counts conducted on Day 30. Dogs in the two-dose groups were examined with an otoscope on Days 14 and 44, and had total mite counts performed on Day 60. For total mite counts, dogs were sedated and each ear was flushed and processed separately. The ear canals were filled with Docusol® (5% docusate sodium) and massaged to loosen the contents. The docusate sodium solution was then removed from the ears and poured through a 38 μm sieve. The ears were then flushed with a warm saline solution which was poured through the same sieve. The ears were examined otoscopically and if needed the flushing process was repeated until the ear canals were assessed as clean (no visible cerumen or mites). The contents of the sieve were rinsed with water and transferred to a Petri dish for examination under a stereo microscope. All live mites (adults, larvae and nymphs) were counted and the counts for the two ears were summed for each animal's total mite count.

2.3. Data analysis

All calculations were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA). The individual dog was the experimental unit. Mite counts were transformed by the $\log_e(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Differences were assessed at the two-sided significance level $\alpha = 0.05$. Geometric means (back transformed means) were calculated from the least squares means. Efficacy (% reduction) was calculated versus the pretreatment count or placebo using the following formula:

$$\%\text{Efficacy} = \frac{(\text{Mean Pretreatment[Placebo]Count - Mean Post-treatment Count})}{\text{Mean Pretreatment[Placebo]Count}} \times 100$$

Live *Demodex* mite counts were analyzed using a mixed linear model for repeated measures; including the fixed effect of treatment, day of study and interaction between treatment and day of

Table 2
Evaluation of the clinical signs of demodicosis for dogs treated with three monthly oral doses of sarolaner or receiving weekly topical applications of imidacloprid plus moxidectin; mean percent body area affected by lesions and the proportions of dogs with the different lesion types present.

		Study day						
		−4	14	29	44	59	74	91
Sarolaner (n = 8)	Body area affected (%)	69	60	26	7	4	2	1
	Casts, scales, crusts	100	100	87.5	75.0	37.5	25.0	12.5
	Comedones, papules, pustules	12.5	25.0	0.0	0.0	0.0	0.0	0.0
	Alopecia	100	100	100	87.5	50.0	12.5	12.5
	Erythema	50.0	25.0	37.5	12.5	25.0	37.5	12.5
Imidacloprid plus moxidectin (n = 7)[a]	Body area affected (%)	66	51	16	8	6	3	2
	Casts, scales, crusts	100	85.7	42.9	28.6	42.9	28.6	14.3
	Comedones, papules, pustules	14.3	14.3	0.0	0.0	0.0	0.0	0.0
	Alopecia	100	100	71.4	57.1	42.9	28.6	28.6
	Erythema	61.4	14.3	14.3	14.3	14.3	14.3	28.6

[a] One dog was excluded from assessment of clinical signs due to concurrent antibiotic treatment.

study. The random effects included batch, block within batch, the interaction between block and treatment within batch and error. In addition for each clinical assessment, the frequency of dogs in each group with no live mites, the frequency distribution of skin lesion severity for each lesion type, and percent change from baseline of the extent of skin lesions were determined.

Total live *Otodectes* mite counts at Day 30 and 60 were analyzed using a mixed linear model including the fixed effect of treatment and random effects of block and error.

3. Results

3.1. Efficacy

Prior to treatment initiation, all dogs included in the *Demodex* study had infestations of mites with geometric mean counts of approximately 250 live mites/dog in each group. Both treatments resulted in a rapid reduction in the numbers of live mites (Table 1) with all post treatment counts (from Day 14 on) significantly lower than the pretreatment counts ($P \leq 0.0001$). This reduction was notably more rapid for the oral sarolaner treatment with mite counts for this treatment being significantly lower than those for the imidacloprid plus moxidectin group on Day 14 ($P = 0.0331$) and Day 29 ($P = 0.0038$). Mean counts were not significantly different on subsequent examinations ($P > 0.05$). The proportions of dogs that were free of live mites in the sarolaner-treated group were 37.5% on Day 14, 75% on Day 29, and 100% on all subsequent days. For dogs receiving weekly topical imidacloprid plus moxidectin, 37.5% were free of live mites on Days 14 and 29, 62.5% on Day 44, 87.5% on Day 59 and all dogs had no live mites detected thereafter (Table 1).

In this study, one imidacloprid plus moxidectin-treated dog was excluded from the assessment of clinical sign progression due to concurrent treatment for an accidental laceration with a systemic antibiotic and non-steroidal anti-inflammatory that could have aided in the resolution of clinical signs of demodicosis. Beginning on Day 14, there was improvement noted in the clinical signs of demodicosis in both treatment groups that continued through all subsequent time points (Table 2). Comedones, papules and pustules had resolved completely by Day 29 in both treatment groups. By the end of the study, casts, crusts and scales had resolved in all but one dog in each treatment group, while alopecia and erythema had resolved in all but one sarolaner-treated dog and two dogs in the imidacloprid plus moxidectin-treated group. Similarly, the average proportion of body surface affected by skin lesions relating to demodicosis was markedly reduced from about 65% pretreatment to 1–2% by the end of the study.

In the *Otodectes* study, qualitative otoscopic exams on Day 14 demonstrated live mites in six of eight placebo-treated dogs, and three of eight sarolaner-treated dogs in the single dose regimen. At 30 days after a single oral treatment, live mites were recovered from seven of eight placebo-treated dogs and only two of the eight sarolaner-treated dogs. The respective geometric mean mite counts were 35.1 and 0.6, resulting in 98.2% efficacy (Table 3). The mean count for the sarolaner-treated group was significantly lower than that for placebo-treated group ($P = 0.0013$). In the two dose regimen, qualitative otoscopic exams on Day 14 demonstrated live mites in four of eight placebo-treated dogs and five of eight sarolaner-treated dogs, and on Day 44 (14 days after the second treatment), revealed live mites in four of eight placebo dogs and none of the sarolaner-treated dogs. At 30 days after two monthly oral treatments, live mites were recovered from five of eight placebo-treated dogs and only a single live mite was found in one of the eight sarolaner-treated dogs. The respective geometric mean counts were 19.1 and 0.1, resulting in 99.5% efficacy (Table 3). The mean count for the sarolaner-treated group was significantly lower than for the placebo-treated group ($P = 0.0195$).

3.2. Treatment

In both studies, all dogs treated with oral tablets were dosed completely; no tablets were expelled and no evidence of emesis was observed in any animal. In the *Demodex* study, all dogs treated with the imidacloprid plus moxidectin solution received the appropriate topical dose, however a negligible amount of runoff was noted on a few occasions. This is not unexpected following application of a topical liquid formulation.

3.3. Health observations

There were no adverse events in any either study that were considered related to treatment with sarolaner.

4. Discussion

The two studies reported here confirmed the systemic acaricidal efficacy of the novel isoxazoline, sarolaner, against two mite species commonly infesting dogs. A single oral dose at 2.0 mg/kg resulted in >97% reduction in *Demodex* mite counts at 14 days and >99% at 29 days with a corresponding improvement in the clinical signs of demodicosis. Following a second monthly treatment, no live mites were recovered from any sarolaner-treated dog. This level of efficacy compared favorably to that of the commercial comparator (topical imidacloprid plus moxidectin) applied at the recommended dosage and greatest frequency, as a dog in that group continued to harbor live mites until Day 59, at which time 11 weekly treatments had been administered.

For dogs with induced aural infestations of *O. cynotis,* sarolaner administered at 2 mg/kg as a single oral dose resulted in a 98.2% reduction and two doses of sarolaner, administered one month apart, resulted in a 99.5% reduction in ear mites compared to placebo-treated controls.

Generalized demodectic mange is a serious, debilitating and often life-threatening disease that may be difficult to treat; available therapies generally require repeated application over lengthy periods and often at off-label dosages. Side effects are not uncommon at effective dose rates and dogs need to be monitored closely (Johnstone, 2002; Bissonnette et al., 2009). This study was conducted with a positive control and no negative control group due to welfare concerns. Thus, the possibility of self-cure in these animals cannot be discounted and effectiveness of treatments may be overestimated in the model and not truly reflect the effectiveness seen in a clinical situation. That said, all dogs included in the study had clinical signs of generalized demodecosis which as a chronic disease is considered unlikely to resolve without treatment (Paradis and Page, 1998). In this study, sarolaner was administered at the minimum dose recommended for month-long control of fleas and ticks and provided effective control of *Demodex* mites (99.8%) following a single dose, with apparent eradication of mites following the second monthly dose with concurrent rapid resolution of clinical signs indicating the potential of sarolaner for convenient treatment of generalized demodicosis. Notably, sarolaner was as, if not more, effective than intensive (weekly) treatment with an approved topical product. The efficacy seen with sarolaner was also comparable to that of off-label, oral ivermectin treatment which has been shown to be more effective than topical moxidectin plus imidacloprid (Paterson et al., 2009, 2014). This potent systemic, miticidal efficacy of the isoxazoline class has also been demonstrated for another compound, fluralaner, which demonstrated similar efficacy after oral administration in dogs with generalized demodicosis (Fourie et al., 2015).

Table 3
Geometric mean total ear mite counts, range and percent efficacy relative to placebo for dogs with induced infestations of *Otodectes cynotis* and treated with either a single oral dose or two monthly doses of a sarolaner.

		n	Count Day	Live mite count		
Treated	Treatment			Mean	Range	Efficacy (%)
Day 0	Placebo	8	30	35.1	0–636	–
	Sarolaner	8	30	0.6*	0–11	98.2
Day 0 and Day 30	Placebo	8	60	19.1	0–1034	–
	Sarolaner	8	60	0.1**	0–1	99.5

* Geometric mean live mite count for sarolaner significantly lower than placebo ($P = 0.0013$).
** Geometric mean live mite count for sarolaner significantly lower than placebo ($P = 0.0195$).

Ear mites are also ubiquitous parasites of dogs and sarolaner administered orally at 2 mg/kg was highly effective against induced infestation after a single dose or two monthly doses. This level of efficacy was as good, or better, than that expected for topically administered products for the treatment of ear mite infestations.

There were no adverse reactions to treatment with oral sarolaner in these studies. This, combined with the excellent efficacy against both *Demodex* spp. and *O. cynotis* mites following one to two monthly treatments, indicates the potential of sarolaner for the convenient and effective treatment of these mite infestations and their associated diseases in dogs.

5. Conclusions

Monthly oral administration of sarolaner at 2 mg/kg to dogs with generalized demodicosis was highly effective in eliminating mites and resolving clinical signs of the disease. Pretreatment mite counts were reduced by 97.1% at 14 days and 99.8% at 29 days after the first dose, with no live mites detected thereafter. All dogs showed marked improvement in the clinical signs of demodicosis. Against an induced infestation of *O. cynotis* in dogs, this dose of oral sarolaner reduced mite counts by 98.2% after a single dose and by 99.5% after two monthly doses.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park, NJ. JJF is an independent investigator contracted for these studies. All the other authors were employees of Zoetis. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

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