Veterinary Parasitology 222 (2016) 62–66

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

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

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ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 17 February 2016
Accepted 25 February 2016

Keywords:
Demodex spp.
Demodicosis
Mange
Ear mites
Otoacariasis
Sarolaner
Dog
Oral

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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http://dx.doi.org/10.1016/j.vetpar.2016.02.027

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)