Research Square

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Efficacy Investigation of a chewable tablet containing sarolaner, moxidectin and pyrantel (Simparica Trio®) in the treatment of Otodectes cynotis in dogs

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Research Article

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Abstract

Background: Otoacariasis is a highly contagious, pruritic infestation of dogs caused by the ear mite *Otodectes cynotis*. Treatment with either moxidectin or sarolaner has been successful in dogs.

Methods: In a dose confirmation study and a clinical field study, the efficacy of a chewable tablet containing sarolaner, moxidectin and pyrantel (Simparica Trio®; Zoetis) against natural ear mite infestation was evaluated. In the dose confirmation study, efficacy was evaluated on Day 14 and 30 after a single treatment at a dose of 1.2 mg/kg sarolaner, 0.024 mg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt). In the field study, efficacy of the commercial dose of Simparica Trio was evaluated as parasitological cure rate after a single treatment and its non-inferiority to the control product (Advocate® spot-on, 100 mg/mL (minimum 10mg/kg) imidacloprid + 25 mg/mL (minimum 2.5 mg/kg) moxidectin; Elanco Animal Health) was evaluated. If mites were still present on Day 30, a second treatment was provided, and efficacy was evaluated on Day 60.

Results: In the dose confirmation study, otoscopy showed no mites in any of the Simparica Trio-treated dogs on Day 14 or Day 30, and a 100% efficacy ($P < 0.0001$) was achieved against *O. cynotis*. In the field study, the parasitological cure rate in the Simparica Trio-treated dogs was 87.1% on Day 14 and 96.8% on Day 30 compared to 75.0% on Day 14 and 83.3% on Day 30 in Advocate spot-on-treated dogs. Three Simparica Trio-treated dogs were treated again on Day 30, resulting in a 100% efficacy on Day 60. The efficacy for Simparica Trio, as determined by parasitological cure rate, was non-inferior to Advocate spot-on solution on both Days 14 and 30.

Conclusions: The results of the dose confirmation and clinical field study demonstrate the safety and high efficacy of Simparica Trio against natural infestations with *O. cynotis* in dogs.

Background

Otoacariasis (otodectic mange) is a highly contagious, pruritic infestation of dogs caused by the ear mite *Otodectes cynotis*, an obligate mite parasite that inhabits the vertical and horizontal ear canals. The life cycle is completed entirely on the host in approximately three weeks, and adults may persist in the ear canal for up to two months [1]. Infested dogs may develop otitis externa characterized by erythema of the vertical and horizontal canals and a dark brown, ceruminous otic exudate; infestations may also extend to the head, neck, tail base, and trunk. Transmission occurs primarily via direct contact between animals [2].

Several liquid aural preparations containing antifungals, antibiotics, corticosteroids, and/or parasiticides are licensed for treatment of *O. cynotis* infestation in dogs; however, these products typically have limited residual activity and require repeated application over multiple days. In contrast, monthly topical spot-on formulations containing macrocyclic lactones (selamectin or moxidectin) have been reported to be effective for treatment and control of *O. cynotis* in dogs [3-5]. More recently, oral isoxazoline products

(fluralaner, afoxolaner, and sarolaner) have also been reported to effectively treat *O. cynotis* infestations in dogs [6-13].

Accordingly, a combination of moxidectin and sarolaner would be expected to provide high efficacy against *O. cynotis* infestation in dogs. Simparica Trio® is an orally administered chewable tablet containing sarolaner (1.2–2.4 mg/kg), moxidectin (0.024–0.048 mg/kg), and pyrantel (5–10 mg/kg). In addition to activity against several nematodes, fleas, and ticks, Simparica Trio has demonstrated efficacy against *Sarcoptes scabiei* var. *canis* [14] and *Demodex canis* [15] in dogs. The objective of the studies reported here was to evaluate the efficacy of Simparica Trio against *O. cynotis* infestation in dogs.

Methods

One dose confirmation study and one clinical field study were conducted to evaluate efficacy of Simparica Trio against *O. cynotis*. Both studies were GCP (Good Clinical Practice) compliant.

Dose confirmation study

The dose confirmation study evaluated the efficacy of a single dose of Simparica Trio, at the minimum recommended dose of 1.2 mg/kg sarolaner, 0.024 mg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt), against *O. cynotis* in naturally infested, client-owned dogs, over a period of 30 days after administration. A total of 18 healthy privately-owned dogs with a confirmed natural infestation of *O. cynotis* were allocated to either the control group (T01, n=9), which received a tablet dietary supplement (ProPlan Purina Multivitamin+; Nestlé Purina PetCare), or the Simparica Trio-treated group (T02, n=9). Treatment was administered once on Day 0, and bilateral otoscopic examinations were performed in all dogs prior to treatment (Day 0) and repeated on Day 14 (±1 day) and Day 30 (±1 day). Additionally, on Day 30 (±1) each ear was flushed separately. The ear canal was filled with Zatalax® (i.e. aqueous solution of vegetal glycerin; Zeta Farmaceutici) and massaged lightly externally until the ear duct content had dissolved. The dissolved solution was flushed, using lukewarm water, into a labelled plastic container, followed by additional flushing using lukewarm saline until the ear appeared clean. After completion of the flushing procedure otoscopic examination on both ears was performed and if persistent cerumen deposits and/or mites were observed the flushing procedure was repeated until both ear canals were judged clean and mite free. Gloves and protective apron/clothing were changed between animals during collection and sample handling. The ear-flushing effluent was passed through a 38 µm sieve. The sieve was rinsed immediately with clean water, and the retained material and remaining container contents were transferred to labeled bottles for subsequent counting. Samples were stored at room temperature until analysis. Mite counts and categorization (larvae, nymphs and adults) were conducted by experienced study personnel on the same day as collection, or at the latest within a day of collection (Figures 1-3).

The primary efficacy variable was calculated based on the percent reduction in live mite counts (larvae, nymphs and adults combined) relative to the negative control at Day 30 (±1) using the following formula:

$$\% \text{ reduction} = 100 \times \frac{[\text{mean count (placebo)} - \text{mean count (treated)}]}{\text{mean count (placebo)}}$$

Total mite counts at Day 30 (± 1) were analyzed using a general linear mixed model. The model included the fixed effect of treatment and random effects for block and error. Treatment Least Square Means (LSM) and standard errors were calculated, and 95% confidence intervals were constructed by treatment. The model was assessed for departure from normality and was natural log transformed ($\log_e\{x+1\}$) prior to analysis. *A priori* contrasts among least squares means for total mite count were used to assess the difference between the negative control and Simparica Trio-treated groups at the 5% level of significance ($P \leq 0.05$). The presence of mites at otoscopic examinations for each treatment was summarized on Day 0, Day 14 (±1) and Day 30 (±1).

Field study

The field study was conducted in two European countries (Italy and France) and aimed to evaluate the efficacy and safety of Simparica Trio at the commercial dose range against *O. cynotis* administered as a monthly treatment for dogs for up to two months. To that end, dogs were enrolled based on the presence of clinical signs consistent with ear mite infestations and with at least one live mite present in at least one ear. If more than one dog in a household met the inclusion criteria, the animal with the most severe ear mite infection was selected as the primary patient in the household. The primary patient was randomised to treatment and was used for efficacy and safety evaluations.

In total, 142 primary dogs were allocated randomly to one of the two treatment groups: Simparica Trio® (T01; n=94) or positive control (imidacloprid/moxidectin (Advocate® spot-on solution; Elanco); T02; n=48). All other dogs in the same household as the primary dog were enrolled as supplementary patients, received the same treatment as the primary dog in the same household and these supplementary dogs were included in the safety assessments. In total, 88 supplementary dogs were enrolled in T01 and 39 in T02. Treatments were administered monthly on Days 0 and 30. Otoscopic examinations of both ears on each primary dog were performed on Days 0, 14 and 30. The otoscopic examination for the presence of viable *O. cynotis* (larvae, nymphs or adults) included the evaluation of the external auditory canals and the pinnae. If live mites were observed on Day 30 in a primary dog, then all dogs in the household were administered a second treatment and an additional otoscopic examination, including evaluation of the external auditory canals and pinnae, was performed on Day 60.

The primary efficacy variable was the parasitological cure rate, defined the % of dogs in the given treatment group having no live mites in the *O. cynotis* assessments on the respective study day. Parasitological cure rates for Days 14 and 30 were analysed using a generalised mixed linear model for repeated measures for a binomial distribution with logit link. The model included fixed effects for treatment, time and treatment by time. The random effects included clinic, block within clinic, the interaction of clinic and treatment and the interaction of clinic, treatment and time, and animal within site and block. Back-transformed least squares means were used as estimates for parasitological cure rates for each treatment at each time point. Non-inferiority of parasitological cure rate for Simparica Trio to Advocate spot-on solution was assessed for primary efficacy at Day 30 (±3) and secondary efficacy at Day 14 (±3) using a margin of 15% at the one-sided 0.025 significance level. The severity of clinical signs of otoacariasis of the primary dogs (including head shaking, pruritus/ear scratching, trauma or alopecia of the pinnae, and erythema, ulceration, and debris in the ear canals) was evaluated on a four-grade scale as absent, mild, moderate or severe prior to treatment on Days 0, 14 and 30, and as applicable on Day 60. The same person made these assessments throughout the study.

Results

Dose confirmation study

Complete dosing was achieved in all dogs, and no concomitant treatments were administered during this study. No abnormal health events were observed following treatment with Simparica Trio in this study, further supporting the product's known safety profile. In the control group, one animal exhibited an abnormal health event at study completion (Day 30), characterized by moderate weight loss that persisted beyond the study period. Serological testing confirmed a concurrent *Ehrlichia canis* infection, and the animal received appropriate treatment. On otoscopic examination, 3 of the control dogs were unilaterally infested and 6 were bilaterally infested at Day 14 (±1). By Day 30 (±1), infestation in control dogs was predominantly bilateral (8 bilateral and 1 unilateral). In the control group, the geometric mean mite count on Day 30 was 30.2 (range 17 to 54). An overview of the individual total ear mite counts on Day 30 is provided in Table 1. In the Simparica Trio-treated group, otoscopy showed no mites in any dog on Day 14 (±1) or Day 30 (±1), and a 100% efficacy ($P < 0.0001$) was achieved against *O. cynotis* in naturally infested dogs in this study.

Field study

There were no treatment-related adverse reactions during the clinical field study. Out of the 93 positive animals enrolled in the Simparica Trio-treated dogs, 12 were positive on day 14 and 3 were positive on Day 30, after a single treatment. These three dogs were negative for live mites after the second treatment (Day 60). The number of ear mite positive animals in the control group (n=48) was 12 on Day 14, 8 on Day 30 and 1 on Day 60 (see also Table 2). The parasitological cure rate of Simparica Trio was 87.1% on Day 14, 96.8% on Day 30 and 100% on Day 60. For Advocate spot-on solution, the parasitological cure rate was 75.0% on Day 14, 83.3% on Day 30 and 87.5% on Day 60. The efficacy for

Simparica Trio was non-inferior to Advocate spot-on solution at both Days 14 and 30. At enrolment the following clinical signs were observed in the indicated percentage of primary dogs in the Simparica Trio treated group: head shaking (97.9%), pruritus/ear scratching (93.6%), trauma or alopecia of the pinnae (26.6%), erythema in the ear canal (69.1%), ulceration in the ear canal (3.2%) or debris in the ear canal (96.8%). Similar percentages of each clinical sign were observed in the Advocate spot-on-treated group in the indicated percentage of primary dogs at enrolment: head shaking (97.9%), pruritus/ear scratching (95.8%), trauma or alopecia of the pinnae (29.2%), erythema of the ear canal (77.1%), ulceration in the ear canal (2.1%) and debris in the ear canal (95.8%). Clinical signs of *O. cynotis* improved throughout the study in both groups. In the Simparica Trio group at study completion, trauma or alopecia of the pinnae was absent in all animals.

Discussion

In the dose confirmation study, naturally infected animals were enrolled in two treatment groups. A single treatment with Simparica Trio at a dose of 1.2 mg/kg sarolaner, 0.024 mg/kg moxidectin and 5 mg/kg pyrantel demonstrated 100% efficacy against natural infestations with *O. cynotis* both on Day 14 and Day 30. In a previous dose confirmation study evaluating the efficacy of Simparica® (sarolaner; Zoetis) against ear mites, 98.2% and 99.5% efficacy were reported after one or two treatments, respectively [8]. The 100% efficacy observed on both Day 14 and Day 30 in this study, might be due to the additive efficacy of moxidectin, despite the lower dose of sarolaner in Simparica Trio. The use of naturally infested animals in a dose confirmation study against this mite is justified by the challenges around induced *O. cynotis* infections, and similar study designs were reported before [7;16]. While the study animals were not maintained under controlled conditions during the study, no other animals (cats or dogs) were in direct contact with the enrolled dogs in any of the households during the 30-day study, preventing the possibility of re-infestation and potential bias of the study results.

In the field study, the inclusion of a total of 142 primary patients through 12 veterinary practices in Italy and 4 in France ensures that the findings are suitably representative of different environmental and climatic conditions and possible variations in susceptibility. In these naturally infected animals, the parasitological cure rate was 96.8% after a single Simparica Trio treatment, confirming the high efficacy observed in the dose confirmation study. As anticipated in the study design, a second treatment might be required in some animals to break the *O. cynotis* life cycle, which is typically 21 days, and protect the dogs from reinfestation [11]. In a limited (3.2%) number of Simparica Trio-treated dogs, a positive ear mite count on Day 30 was observed, and these dogs were re-treated on Day 30 to ensure they were mite negative on Day 60. The requirement to provide a second isoxazoline treatment to a limited number of animals is in line with previous studies by the lower than 100% efficacy after a single fluralaner [7] or multiple afoxolaner [11] treatments. In this field study, a 100% parasitological cure rate was achieved after the second treatment. In terms of clinical symptoms, a marked reduction in clinical signs associated with *O. cynotis* was also observed at study completion, further confirming the positive impact of treatment.

Conclusion

The results of the dose confirmation and clinical field study demonstrate the safety and high efficacy of Simparica Trio against natural infestation with *O. cynotis* in dogs.

Abbreviations

O. cynotis – Otodectes cynotis

Declarations

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Author Contributions

TG: Conceptualization, methodology, writing—original draft preparation, writing—review and editing.

AL: Conceptualization, Validation, Project Administration, Funding acquisition, Data curation, writing—review and editing

LT: Conceptualization, Formal analysis, writing—review and editing

GV Conceptualization, Validation, Data curation, Resources, writing—review and editing

GM: Investigation, writing—review and editing

FV: Investigation, writing—review and editing

All authors read and approved the final manuscript.

Availability of data and materials

Data can be requested by contacting the corresponding author.

Ethics approval and consent to participate

The study protocols were reviewed and approved by the study site's Institutional Animal Care and Use Committee prior to implementation. Both studies involved client-owned animals and informed consent was obtained from all owners before their animals participated in the study.

Consent for publication

Not applicable.

Competing interests

Authors TG, AL and LTwere employees of Zoetis at the time of the conduct of the studies.

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Tables

Table 1. Dose confirmation study: Summary of individual total ear mite counts at Day 30, with individual and mean mite counts, and % reduction

Treatment	Animal	Mite Count	Treatment	Animal	Mite Count
Control Group	IA01	17	Simparica Trio®	IA02	0
	IA04	48		IA03	0
	IA06	54		IA05	0
	IA07	33		IA08	0
	IA09	22		IA10	0
	IA11	30		IA12	0
	IA13	32		IA14	0
	IA15	33		IA16	0
	IA18	20		IA17	0
Arithmetic Mean		32.1	Arithmetic & Geometric Mean		0.0
Geometric Mean		30.2	% Reduction		100

Table 2. Field study: Number of Dogs with Live

Treatment	Visit	Live mites present	
		Number of dogs	% of dogs
Simparica Trio®	Day 0	93	100
	Day 14	12	12.9
	Day 30	3	3.2
	Day 60	0	0
Advocate® Spot-on	Day 0	48	100
	Day 14	12	25.0
	Day 30	8	16.7
	Day 60	1	12.5

Figures

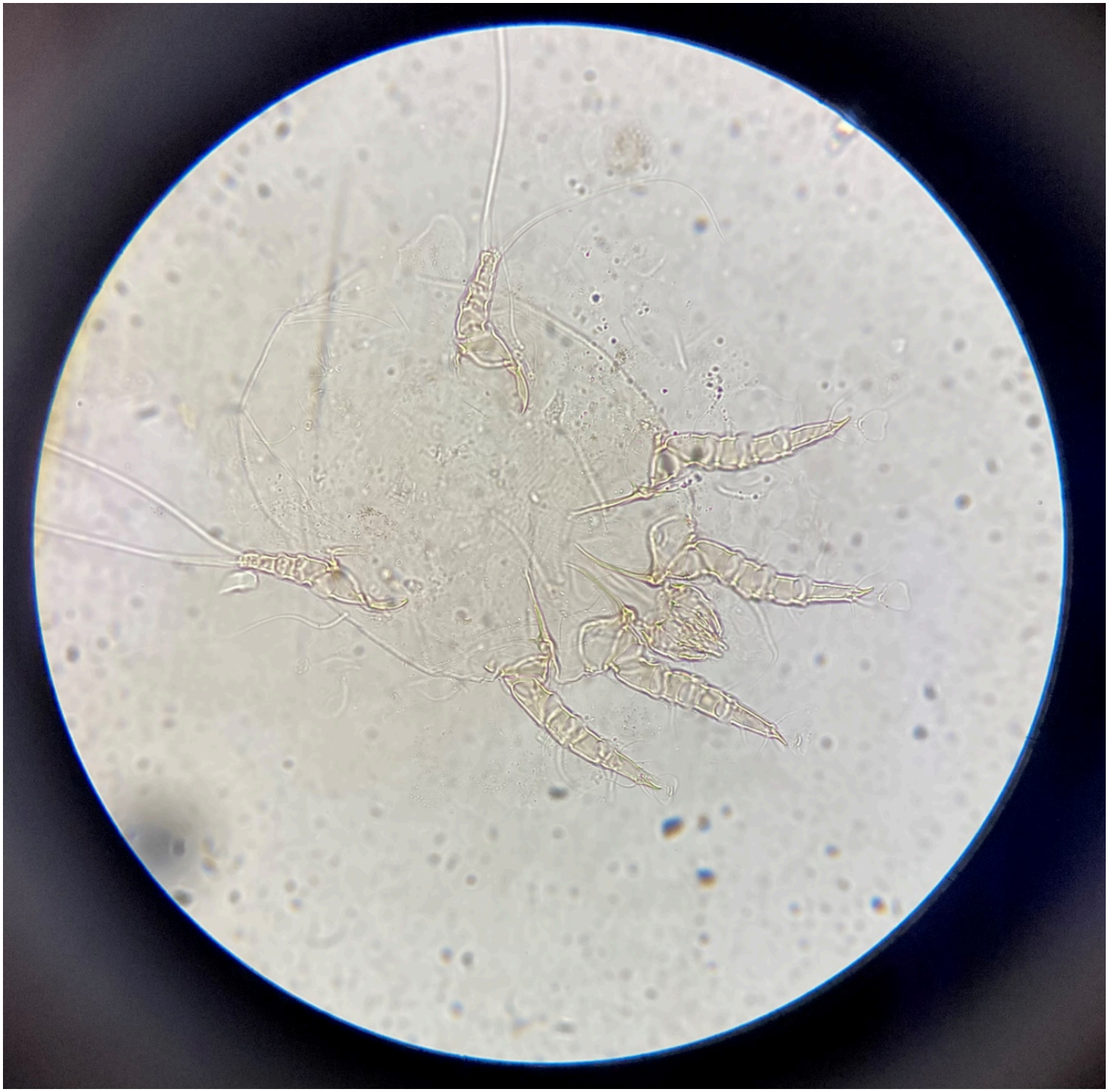


Figure 1

Otodectes cynotis larva. Optical microscope, 40x.



Figure 2

Adult female *Otodectes cynotis*: detail of the anterior region and gnathosoma. Optical microscope, 40x.



Figure 3

Adult male *Otodectes cynotis*: detail of the posterior region and male genital organs. Optical microscope, 40x.