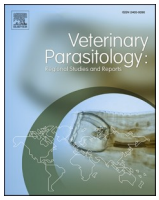
Veterinary Parasitology: Regional Studies and Reports 59 (2025) 101228

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

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



Short Communication

Treatment of notoedric mange with esafoxolaner, eprinomectin and praziquantel in naturally infested cats under in-home conditions

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ARTICLE INFO

Keywords:
Cats
Ectoparasiticide
Efficacy
Esafoxolaner
In-home conditions
NexGard® combo
Notoedres cati
Notoedric mange

ABSTRACT

Notoedres cati is a contagious, burrowing mite infesting cats that causes mange with intense pruritus and dermatological clinical signs. The aim of the current study was to assess the efficacy of a single administration of NexGard® Combo (esafoxolaner, eprinomectin and praziquantel) against *N. cati* in naturally infested cats under home conditions. A total of 17 domestic shorthair cats naturally infested with *N. cati* that were living in the same house were randomly assigned to either the treatment group ($n = 9$) receiving NexGard® Combo at a dosage of 0.12 mL/kg of their body weight according to label instructions or a negative control group ($n = 8$) receiving mineral oil at 0.12 mL/kg (Day 0). The cats were separated into different rooms with no contact between the two groups in the same household. Clinical scores, mite counts from skin scrapings and body weights were evaluated on Days −3, 0, 14, 28, 42, and 56. Descriptive statistics (means ± standard deviations) were calculated, and a mixed general linear model was used for statistical analysis. NexGard® Combo showed 100 % efficacy against *N. cati,* and no live mites were found from Day 14 until the end of the study. Treated cats also exhibited lower clinical scores and higher body weights than the control group by Day 56 ($p < 0.05$), while placebo-treated cats displayed high mite counts and clinical scores throughout the study. None of the cats displayed any adverse events related to treatment. These findings highlight the efficacy of the combination of esafoxolaner, eprinomectin and praziquantel in treating notoedric mange in cats under in-home conditions.

1. Introduction

Notoedres cati (Hering, 1838) is an obligate, burrowing mite (Order: Sarcoptiformes, Family: Sarcoptidae) that typically infests cats and other felids but can also be found in dogs and sometimes in humans posing a threat to public health (Beugnet et al., 2018). All stages of *N. cati* (one larval, two nymphal and the adults) occur on the host, with the life cycle taking approximately 2 to 3 weeks to complete (Leone and Siew Han, 1996). The morphology of the mites is quite similar to *Sarcoptes scabiei*, with adult females of *N. cati* laying their eggs inside tunnels they dig in the layers of the epidermis (Deplazes et al., 2016). The disease is highly contagious, with transmission occurring mainly via direct contact between hosts and secondarily by contact with contaminated fomites from the environment (Deplazes et al., 2016; Beugnet et al., 2018), with the mites surviving just 2–3 days without a host (Deplazes et al., 2016). Furthermore, although mites can infest humans, they cannot complete their life cycle in this host, and thus infestations are self-limited (Beugnet et al., 2018).

Notoedric mange is typically located in the head, face, ear pinnae, eyelids and neck, with clinical signs including intense itching, papules, hyperkeratosis, crusting, desquamation, alopecia lesions due to scratching, with the affected areas becoming lichenified (Foley et al., 2016; Blazejak et al., 2023). Without treatment, symptoms quickly expand to the legs, perineum and then throughout the cat's body, while the uncontrolled self-scratching can often result in subsequent bacterial or fungal infections (Foley et al., 2016; Blazejak et al., 2023). In severe cases, infestations can lead to lethargy, reduced appetite, weight loss and even death (Leone and Siew Han, 1996).

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https://doi.org/10.1016/j.vprsr.2025.101228
Received 15 November 2024; Received in revised form 12 February 2025; Accepted 17 February 2025
Available online 18 February 2025

Notoedric mange is distributed worldwide (Baker et al., 2021), with a few epidemiological surveys in cats referring to prevalence values varying from 0.6 % in Israel (Salant et al., 2014), 1.3 % in the U.S.A. (Wyrosdick et al., 2017), 1.98 % in Slovenia (Rataj et al., 2004), and 2.35 % in Greece (Lefkaditis et al., 2015). Local outbreaks are expected, with kittens, homeless cats, cats living in groups/shelters, cats with a poor body condition score and those immunocompromised or suffering from comorbidities having a higher infestation risk (Leone and Siew Han, 1996; Deplazes et al., 2016).

Diagnosis can be achieved by visualising the adult mites (0.2–0.4 mm long) under a stereomicroscope after deep scraping the affected areas with a scalpel blade until bleeding of the skin capillaries and placing the skin material on a microscope slide (Beugnet et al., 2018).

Treatment options traditionally consisted of administrating macrocyclic latones (selamectin, eprinomectin, moxidectin) in spot-on formulations or fipronil spraying (Itoh et al., 2004; Hellmann et al., 2013; Knaus et al., 2014; Deplazes et al., 2016; Beugnet et al., 2018). In recent years, topical formulations containing an isoxazoline (esafoxolaner) or a bispyrazole (tigolaner) have also proven effective in treating notoedric mange in cats (Baker et al., 2021; Blazejak et al., 2023). Contemporaneously, all other animals living with the infested cat should also be treated (Leone and Siew Han, 1996). Supportive measures comprise bathing using a shampoo with anti-seborrhoeic properties to eliminate crusting, thoroughly cleaning and disinfestation of the cat's environment (Beugnet et al., 2018; Blazejak et al., 2023). A parasitological cure is achieved when no more mites are found in the dermal scrapings, and a clinical cure is achieved with the resolution of symptoms.

NexGard® Combo (Boehringer Ingelheim Animal Health, Toulouse, France) is a commercially available spot-on formulation containing the isoxazoline esafoxolaner, as well as eprinomectin and praziquantel, and is an endectoparasiticide effective against a plethora of ectoparasites (Beugnet, 2021). These include fleas, ticks and mites like *Otodectes cynotis* (Beugnet, 2021). The aim of the current study was to assess the efficacy of a single administration of NexGard® Combo against *N. cati* in naturally infested cats under in-home conditions.

2. Materials and methods

2.1. Compliance and ethical approval

The current study conformed to the standards of good clinical practice (VICH Guideline 9) (The European Agency for the Evaluation of Medicinal Product, 2000). Furthermore, the research was approved by the Ethics Committee of the Aristotle University of Thessaloniki (326,409/2023) and was conducted in compliance with Greek laws concerning animal care, while all cats were treated with regard to their welfare. Written informed consent was obtained by the cats' owner to participate in the study.

2.2. Study animals

In total, a pack of 17 domestic shorthair cats (7 males and 10 females) naturally infested with *N. cati,* based on pre-treatment skin scrapings, were included in the current study. All animals were living together in the same household in contact with each other, and shared the same beddings, enabling transmission of the parasite. The study took place in Greece from March until May 2024 and was a single-centre, blinded, randomised study including negative controls with a parallel group design carried out under in-home conditions. All cats were client-owned, 1–5 years old, with weights ranging from 2.5 to 3.7 kgs before treatment. Each group was housed separately throughout the study period in a monitored separate room of the owner's house, without outdoor access and was already acclimatised before the study's initiation. Each room was approximately 20m^2 and cleaning was performed with a vacuum cleaner every week. For the duration of the study, water was provided ad libitum, and the cats were kept on their regular feed schedule while their overall health was monitored daily. None of the cats had any clinical signs besides those associated with notoedric mange, nor were they pregnant or lactating. Additionally, the cats had not been treated for ectoparasites at least 6 months before the study's initiation.

2.3. Study design

With Day 0 (D0) marking the treatment administration, physical examinations, notoedric mange clinical scoring, dermal scrapings and live mite counting were carried out on D-3 and then on D14, D28, D42 and D56. Each cat was weighed on D0 before treatment, on D28 and D56 to determine any fluctuations in body weight over the study. The scale used was verified with standard weights before weighing the cats on the respective days.

Cats were randomly assigned to one of the two groups according to their sex, mite counts and clinical scores at the beginning of the study, with effort put into the randomisation so that the two groups were similar. The two groups were the treatment group (Nexgard® Combo) and the negative control group (mineral oil - placebo treatment).

Each study personnel had a distinct role to ensure blinding, with those blinded performing weighing, clinical examinations, clinical scores, dermal scrapings, and live mite counts, unaware of the treatment groups. On the other hand, non-blinded individuals were only responsible for dispensing treatments without getting involved in the diagnostic or clinical examinations.

2.4. Treatment administration

Cats in the treatment group received a single spot-on treatment (NexGard® Combo) of 0.12 mL/kg of their weight, which corresponds to the lowest dosage of 1.44 mg of esafoxolaner, 0.48 mg of eprinomectin, and 10.0 mg of praziquantel per kg of bodyweight, according to the product's label. Each cat's hair was separated for the application, and the spot-on was applied once on D0 straight to the cat's skin, in a single location along the neck's midline, between the shoulder blades and base of the head. The cats in the negative control group received mineral oil (paraffin oil) in the same areas with the same volume dosages (0.12 mL/kg of their bodyweight). Besides daily health observations, the cats were observed for 3 h post-administration for any adverse events.

2.5. Assessment of live mite counts and clinical scores

Dermal scrapings with a scalpel blade were performed on the borders of lesions (three surface areas, each 1 cm × 1 cm) on D-3 and the same three areas for the follow-up visits on D14, D28, D42 and D56. Consequently, all samples were taken to the Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, for additional processing, and the mites were identified and enumerated according to their morphological features (Leone and Siew Han, 1996) under a stereomicroscope (Olympus, Research Stereomicroscope System SZH10, Hamburg, Germany).

Regarding the notoedric mange clinical scoring, the type and severity of the notoedric mange lesions and associated clinical signs were assessed and added to each cat's individual clinical score (Table 1; Fig. 1) (Knaus et al., 2014).

2.6. Statistical analysis

The software program SPSS v23 was used for the statistical analyses. Descriptive statistics (means ± standard deviations) were calculated, and a mixed general linear model was used to estimate the fixed effects of treatment, time and their interaction term, and sex, as well as the random effect of the cat on the clinical score, mite counts, and body weight, as described below (Model 1):

Table 1
Notoedric mange clinical scoring.[1]

Clinical Score	Severity	Lesions & Clinical Signs
0	Healthy skin	Normal skin
1	Regression signs	Easy lifting and detachment of crusts, incomplete hair growth, and possible or absent mild pruritus
2	Mild Symptoms	Localised lesions, mild alopecia, mild pruritus, and thickening of the skin
3	Moderate symptoms	Lesions localised in the head, alopecia, pruritus, thick skin, possible exudate
4	Severe symptoms	Lesions located in the head and other areas, severe alopecia, intense pruritus, thick skin, crusting, scabbing, possible exudate

[1] Cats with a clinical score of 0 or 1 were regarded as clinically recovered.

Fig. 1. Cat with notoedric mange lesions.

$$y_{ij} = \beta0 + \beta1 \cdot X1_i + \beta2 \cdot X2_{ij} + \beta3 \cdot X3_i + \beta4 \cdot (X1 \cdot X2)_{ij} + u_i + e_{ij} \quad (1)$$

where: y_{ij} = the dependent variable (clinical score, mite counts, body weight) for the i^{th} cat at the j^{th} time-point; $\beta0$ = intercept; $X1_i$ = the fixed effect of the treatment group (2 levels, control and treated group); $X2_{ij}$ = the fixed effect for the time-point [five levels for clinical score and mite counts (D-3, D14, D28, D42, and D56) and three levels for body-weight (D0, D28, and D56)]; $X3_i$ = the fixed effect of sex (2 levels, female and male); $(X1 \cdot X2)_{ij}$ = the fixed effect of the interaction term of the treatment group by the time-point; u_i = the random effect of the cat; e_{ij} = the residual error term.

Heterogeneous first-order autoregressive (ARH1) was used as the most appropriate repeated covariance type, utilising Akaike's Information Criterion (A.I.C.). Statistical significance was set at the 0.05 level.

3. Results

All 9 of the treated cats tolerated the treatment well, and no adverse events were reported. The mean clinical scores, mite counts and body weights, as well as their standard deviations for the treated ($n = 9$) and control ($n = 8$) groups at D-3, D0, D14, D28, D42, and D56, are summarised in Table 2.

The mixed general linear model (Degrees of freedom, F-values, and statistical significance of the factors) is summarised in Table 3.

The analysis revealed a significant difference of the treatment group on the clinical score ($P < 0.001$), the mite counts ($P < 0.001$), and the body weight ($P < 0.05$). In particular, cats in the control group had increased clinical scores and mite counts by approximately 3.35 (95 % CI, 3.05 to 3.65) and 40.0 mites (95 % CI, 35.0 to 44.9 mites), respectively, and decreased body weight by approximately 0.46 kg (95 % CI, 0.20 to 0.73 kg). Moreover, a significant effect of time was observed for the clinical score ($P < 0.001$) and the mite counts ($P < 0.01$) but not for the body weight ($P = 0.184$). In contrast, the effects of the interaction between time and treatment group were significant for all the outcome variables ($P < 0.001$, in all cases), indicating that their changes over time significantly differed between the control and the treated groups. Finally, sex significantly affected both the clinical score ($P < 0.05$) and the body weight ($P < 0.001$) in the control group, but not the mite counts; female cats had a clinical score increased by 0.32 (95 % CI, 0.03 to 0.61) and a bodyweight decreased by 0.77 kg (95 % CI, 0.59 to 0.96 kg).

The mean clinical score, mean number of mites and mean body

Table 3
Degrees of freedom, F-values, and statistical significance of the factors forced into the general linear model.

Dependent Variable	Source	Numerator df	Denominator df	F-value	*p*-value
Clinical score	Group	1	18.14	58.80	<0.001
	Sex	1	22.17	5.29	0.031
	Time	4	40.19	26.49	<0.001
	Time*Group	4	40.19	34.68	<0.001
Mite counts	Group	1	14.61	79.15	<0.001
	Sex	1	27.81	2.00	0.168
	Time	4	37.48	10.99	<0.001
	Time*Group	4	37.48	10.94	<0.001
Body weight	Group	1	13.61	6.38	<0.05
	Sex	1	17.01	78.01	<0.001
	Time	2	21.70	1.83	0.184
	Time*Group	2	21.70	13.37	<0.001

Table 2
Means and standard deviations for the NexGard® Combo treated andControl groups at D-3, D0, D14, D28, D42, and D56.

			Mean (± standard deviation)					
Trait	Group	Sex	D-3	D0	D14	D28	D42	D56
Clinical score (0–4)	C	M	3.0 (1.00)	NA	3.3 (0.58)	3.3 (0.58)	3.0 (0.00)	3.0 (0.00)
	T		2.8 (0.50)	NA	2.0 (0.00)	1.8 (0.50)	1.0 (0.00)	0.0 (0.00)
	C	F	3.0 (0.71)	NA	3.2 (0.84)	3.4 (0.89)	3.6 (0.55)	3.6 (0.55)
	T		3.4 (0.89)	NA	2.6 (0.89)	2.0 (0.71)	1.2 (0.45)	0.0 (0.00)
Mite counts (n)	C	M	41.7 (13.01)	NA	39.7 (9.07)	52.7 (10.02)	44.3 (9.45)	47.7 (10.02)
	T		32.8 (20.87)	NA	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	C	F	17.8 (7.69)	NA	25.6 (9.74)	32.4 (6.88)	34.8 (7.05)	35.0 (5.34)
	T		23.4 (7.02)	NA	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
Body weight (kg)	C	M	NA	3.5 (0.35)	NA	3.4 (0.26)	NA	3.2 (0.21)
	T		NA	3.5 (0.19)	NA	3.5 (0.22)	NA	3.6 (0.32)
	C	F	NA	2.7 (0.11)	NA	2.6 (0.22)	NA	2.6 (0.23)
	T		NA	2.8 (0.15)	NA	2.9 (0.23)	NA	3.2 (0.22)

Group: C = Control, T = NexGard® Combo treated; Sex: M = male, F = female; NA: Measurements are not available.

weight for the two groups over the study's duration are illustrated in Figs. 2, 3 and 4, respectively. After the end of the study (D56), cats belonging to the control group were also rescue-treated with NexGard® Combo according to their body weight in light of the high efficacy in the treated cats.

4. Discussion

All cats in the treatment group had 0 mite counts from D14 until the end of the study, with NexGard® Combo displaying 100 % efficacy against *N. cati* infested cats under natural, in-home conditions. Simultaneously, treated cats had lower (3.35, $P < 0.001$) clinical scores and significantly higher (0.46 kg, $P < 0.001$) body weights by the end of the study compared to cats in the control group, with clinical signs having regressed and all treated cats showing healthy skin on D56 (Knaus et al., 2014). Meanwhile, the mean clinical scores in the control group stayed relatively stable during the study, and the mean mite counts increased until D28 when they reached a plateau.

Regarding the mean mite counts before the study's initiation on D-3, they were comparable with mite counts reported in other studies with naturally-infested cats (Hellmann et al., 2013; Knaus et al., 2014, 2021). The fluctuation in the mean mite counts in the control group from D-3 until D56 has also been observed in previous studies (Knaus et al., 2014, 2021) and may be attributed to the challenging detection of *N. cati* mites, particularly in light to moderate infestations (Knaus et al., 2021). However, none of the cats in the control groups became negative for *N. cati* at any point during the study, and none of them showed a regression of clinical signs, unlike laboratory-controlled studies where the living conditions are highly regulated and a few cats may have 0 mites at certain time points (Knaus et al., 2021). Interestingly, female cats in the control group of the current study had significantly higher clinical scores than males by the end of the study, suggesting a potential susceptibility of female cats to notoedric mange. Further research with more cats is necessary to shed light on the role of cat gender in the susceptibility or resilience against *N. cati*.

Nevertheless, the high efficacy (100 %) of NexGard® Combo in the current study highlights the treatment's effectiveness under natural, under in-home conditions, which more closely resemble the conditions most owners treat their cats, unlike laboratory studies. Another benefit of the combination of esafoxolaner, eprinomectin and praziquantel treatment was the increased weight in treated cats. The weight gain can be attributed to either lower stress for the cats, return to normal appetite,

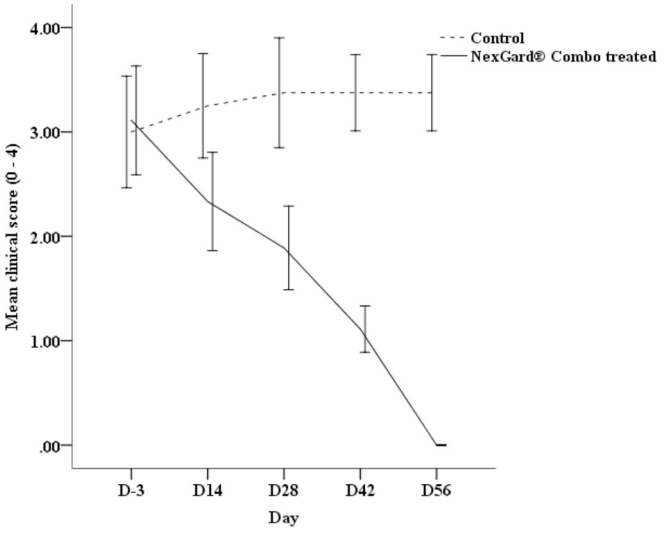

Fig. 2. Mean clinical scores per study group (NexGard® Combo treated and Control) at D-3, D0, D14, D28, D42, and D56. The vertical bars indicate the standard deviations.

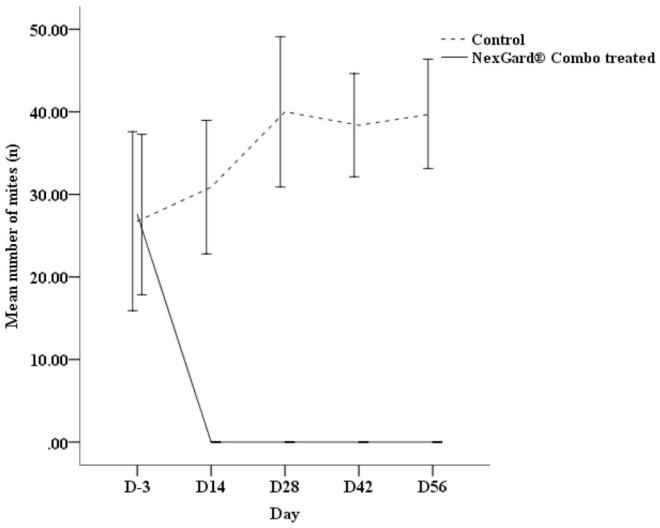

Fig. 3. Mean mite counts per study group (NexGard® Combo treated and Control) at D-3, D0, D14, D28, D42, and D56. The vertical bars indicate the standard deviations.

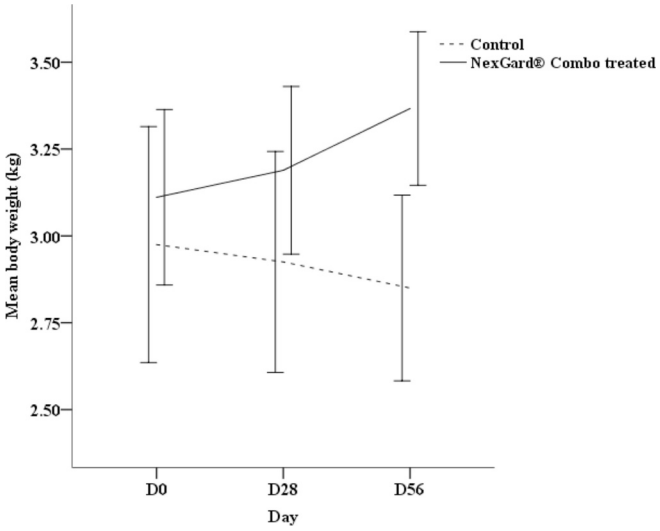

Fig. 4. Mean body weights per study group (NexGard® Combo treated and-Control) at D-3, D0, D14, D28, D42, and D56. The vertical bars indicate the standard deviations.

and overall improved welfare or due to the treatment of other parasites the cats may have been infected with (e.g., roundworms, hookworms, or tapeworms) (Beugnet, 2021), alongside the notoedric mange symptoms resolution. Contrastingly, the weight of cats in the control group showed a steady decline, in accordance with the observed decreased appetite due to *N. cati* infestation (Deplazes et al., 2016).

During the last few years, veterinarians and cat owners have shown their preference for easier-to-use and novel parasiticides (Selzer and Epe, 2021), with NexGard® Combo covering these needs both for endoparasites and ectoparasites (Beugnet, 2021). In addition, the wide range of ectoparasites covered and the convenient monthly use as a spot-on formulation, combined with the high margin of safety (Tielemans et al., 2021), make this compound an ideal choice for treating *N. cati* among fleas, ticks, and other mites (Beugnet, 2021).

5. Conclusions

Following a single spot-on treatment, the combination of

esafoxolaner, eprinomectin and praziquantel (NexGard® Combo) displayed high efficacy (100 %) against *N. cati* in naturally infested cats under in-home conditions. The improved clinical signs, zero mite counts and increased weight in treated cats after 56 days indicate less stress and overall improved welfare, while no adverse events were recorded, making NexGard® Combo a safe and effective treatment against notoedric mange.

Funding

This research received no external funding.

Animal welfare statement

This animal study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Aristotle University of Thessaloniki (326409/2023).

Informed consent statement

Written informed consent has been obtained by the cats' owner to publish this paper.

Ethical statement

This animal study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Aristotle University of Thessaloniki (326,409/2023).

CRediT authorship contribution statement

Georgios Sioutas: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Athanasios I. Gelasakis:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Data curation. **Frederic Beugnet:** Writing – review & editing, Validation. **Elias Papadopoulos:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

Frederic Beugnet reports a relationship with Boehringer Ingelheim Animal Health that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All authors are thankful to the cats' owner for cooperating, providing us with the information needed, and allowing us to publish the present study. The authors would also like to thank Gerolymatos International S. A. for kindly donating the NexGard® Combo products used in the current study.

Data availability

The data presented in this study are available in the main text.

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