



Research paper

Efficacy of afoxolaner for the treatment of ear mite infestation under field conditions

Rossella Panarese^a, Roberta Iatta^a, Riccardo Paolo Lia^a, Wilfried Lebon^b, Frederic Beugnet^b, Domenico Otranto^{a,c,*}

^a Department of Veterinary Medicine, University of Bari, Valenzano, Italy

^b Boehringer Ingelheim Animal Health, Lyon, France

^c Faculty of Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran

ARTICLE INFO

Keywords:

Otodectes cynotis

Ear mite

Dogs

NexGard®

Afoxolaner

Treatment

Malassezia pachydermatis

ABSTRACT

Otodectes cynotis is a commonly occurring surface mite that can be easily transmitted between suitable hosts, including dogs, causing otocariosis. The activity of the systemic insecticide afoxolaner against *O. cynotis* has been tested once under experimental conditions, showing a high efficacy. The present study aimed to i) assess the efficacy of two consecutive monthly oral administrations of afoxolaner (NexGard®) against *O. cynotis* in naturally infested dogs under field conditions and ii) evaluate its impact in reducing bacteria or fungal secondary infections. Dogs, positive for *O. cynotis* (n = 20), were included in the study and allocated in two groups of ten animals each (G1, control group, and G2, treated group). The first group of ear mite-infested dogs was treated with a placebo, while afoxolaner was administered orally to the second group of dogs at Day 0 (D0) and Day 30 (D30), following label instructions. Otoloscopic assessments, deep-swab method and swab samples were performed on all dogs (Days 0, 30, 42) to evaluate the presence or absence of live mites and their number throughout the study, as well as to conduct bacterial and fungal assessments. No adverse events likely related were recorded throughout the study. By Day 42 (D42), all dog's ears were flushed to recover ear mites. All treated dogs became negative, as well as two dogs of the control group. The treatment efficacy of afoxolaner was 100 % based on the arithmetic means of the live mite counts. The clinical scores did not change significantly in the control group, whereas they significantly improved in the treated one from D0 to D30 (p-value = $5.47 \cdot 10^{-5}$). No live mites were present in the afoxolaner-treated group at D42 (p-value = 0.00073). In this field study, two oral administrations of afoxolaner at the recommended dose allowed a complete cure of the infestation. Bacterial and *Malassezia pachydermatis* infections were detected in both groups, although no significant trend was associated to the ear mite treatment.

1. Introduction

Otodectes cynotis (Acari, Psoroptidae) is a common obligatory, non-burrowing mite, causing external otitis in cats, dogs, ferrets, foxes, and, occasionally, humans (Otranto et al., 2004). This mite lives mainly in the ear canal of the host, although it can rarely migrate on other parts of the body (e.g., head, feet, and tip of the tail) (Curtis, 2004). The life cycle lasts three weeks, occurs entirely within the animal's ear, and includes four developmental stages (i.e., egg, larvae, proto- and deutonymph, and adult) (Sweatman, 1958). The ear-mite infestation (i.e., "otocariosis") is a very contagious and worldwide distributed parasitosis mainly affecting young individuals (Taenzler et al., 2017), commonly

diagnosed during routine veterinary physical examinations. Ear-mites primarily feed on desquamated epithelial cells and aural exudates, and eventually pierce the ear lining and feed on tissue fluids (Mullen and Oconner, 2002). The consequent otitis is characterized by vertical and horizontal ear canal erythema with a characteristic dark brown, ceruminous auricular exudate (Taenzler et al., 2017). Papulocrustous lesions (miliary dermatitis) may be also found on the head, feet, and tip of the tail (Scott and Horn, 1987). The intense pruritus may result in self-mutilation, bleeding, and aural haematoma development (Kraft et al., 1988; Baker, 1999). Occasionally, the infestation leads to secondary bacterial infection, possibly resulting in purulent external otitis (Arther et al., 2015). Indeed, pathogens that may be involved and,

* Corresponding author at: Department of Veterinary Medicine, University of Bari, Valenzano, 70010, Bari, Italy.

E-mail address: domenico.otranto@uniba.it (D. Otranto).

<https://doi.org/10.1016/j.vetpar.2021.109607>

Received 31 August 2021; Received in revised form 19 October 2021; Accepted 20 October 2021

Available online 29 October 2021

0304-4017/© 2021 Elsevier B.V. All rights reserved.

consequently, complicate the otocariosis are bacteria of the genera *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli*, as well as yeasts belonging to *Malassezia* genus (Nardoni et al., 2014). However, the ear-mite treatment may play a role in balancing the host immune-response, by eliminating the major pathogen (i.e., ear mites) and allowing the self-cure of the animal from secondary yeast and bacteria infections (Tarallo et al., 2009; Nardoni et al., 2014). Treatment of otocariosis includes the mechanical cleaning of the ear canal followed by either the application of topical products containing acaricides, antibiotics, and synthetic corticosteroids (Pappas and Kartz, 1995; Scherk-Nixon et al., 1997) or the use of systemic acaricidal formulations of macrocyclic lactones (e.g., eprinomectin, moxidectin, selamectin) or isoxazolines (e.g., esafloxolaner, fluralaner, sarolaner) (Shanks et al., 2000; Beugnet et al., 2014; Tielemans et al., 2021). Recent efficacy studies, especially concerning isoxazolines, have been conducted in cats, and an esafloxolaner formulation is registered to treat ear mange in cats in European Union (Tielemans et al., 2021). Few data are available regarding their use in ear mite infested dogs. The oral administration of afoxolaner at the minimum dose of 2.5 mg/kg (Nex-Gard®, Boehringer Ingelheim Animal Health) showed its efficacy in protecting dogs against flea, tick and mite (i.e., *Sarcoptes scabiei* and *Demodex canis*) infestations (Halos et al., 2014; Shoop et al., 2014; Beugnet et al., 2016a, b; Hampel et al., 2018; Lebon et al., 2018). Only a single study using experimentally infected dogs assessed its efficacy against ear mites (Carithers et al., 2016). Therefore, based on these observations, the present study was undertaken to i) assess the efficacy of two consecutive oral monthly administrations of afoxolaner following label recommendations in dogs naturally infested with *O. cynotis*, and ii) evaluate the effect of the ear mite treatment in reducing bacterial or fungal secondary infections.

2. Material and methods

2.1. Animals

Twenty privately-owned dogs (≥ 2 kg and ≥ 8 weeks old), presenting ear mange (≥ 1 live mite in at least one ear) were included in the study. Any dogs with an inappropriate health status, in pregnancy or lactation, or treated with an ecto- or endoparasiticide product within its efficacy duration were excluded.

2.2. Study design

This study was a multi-site (i.e., Apulia region) negative control, blinded, clinical efficacy field trial, using a randomized block design based on the order of animal inclusion, and all evaluations of efficacy were performed by personnel in blinded conditions. The day of enrolment represented by Day 0 (D0) was different for each animal. Each dog was considered as an experimental unit and its allocation in the treatment or control group was performed by the treatment dispenser. To prevent potential bias or unmasking, the treatment assessments were

Table 1

Untreated control group and afoxolaner-treated group (2.5 mg/kg on Days 0 and 30): dog details at the enrolment (D0).

Dog N	Breed	Age (Months)	Sex	Weight (Kg)	Dog N	Breed	Age (Months)	Sex	Weight (Kg)
1	Pointer	84	F	19.0	1	English Setter	132	F	14.0
2	Breton	36	F	15.0	2	Pointer	72	F	18.0
3	Breton	120	F	15.3	3	Breton	5	F	15.0
4	Segugio	3	F	7.7	4	Segugio	54	M	16.1
5	Segugio	3	F	7.4	5	Segugio	60	F	16.1
6	Segugio	6	F	12.6	6	Segugio	30	M	15.0
7	Barboncino	6	F	3.1	7	Segugio	6	F	14.7
8	Barboncino	60	F	3.9	8	Segugio	6	F	14.4
9	Barboncino	48	F	3.5	9	Barboncino	36	F	2.3
10	Barboncino	36	F	4.2	10	Barboncino	24	F	3.3
Arithmetic mean		37.9		8.8			42.5		12.5

conducted in a non-systematic order. The ratio between untreated (Control Group, G1) and treated (Treated Group, G2) animals was 1:1 (Table 1). Health status and otoscopic examinations followed by a semi-quantitative deep swab method and ear swab samples for mycological and bacteriological analyses were performed for all dogs of both groups at D0, D30 \pm 2 (+30 days post-enrolment) and D42 \pm 2 (+42 days post-enrolment). In addition, during the whole study period, all dogs were clinically observed by their respectively owners for possible adverse events due to the product administration.

2.3. Treatment

Animals in G1 and G2 were treated at D0 and D30 \pm 2 with a sham dose (i.e., animals were handled as they were going to be treated), or a chew of NexGard® (afoxolaner oral formulation, delivering a minimum of 2.5 mg/kg) according to the label instructions, respectively. Any dog still positive for *O. cynotis* on D42 \pm 2 were treated with afoxolaner at the end of the study. Dogs were weighed before each treatment for appropriate dosage determination.

2.4. Ear mite assessments

A qualitative evaluation of the ear mite infestation status was performed by otoscopic examination for each dog at each time point (i.e., D0, D30, D42) to determine the presence or absence of visible live ear mites (adult or immature). At the same time, semi-quantitative assessments by deep swab method were also conducted to quantify them. After sampling, the swabs were placed in 60 \times 15 mm Petri dishes and immediately delivered to the laboratory. The mites were counted and identified, following the morphological description of Sweatman (1958). Data regarding number of mites for each ear, sign of pruritus and quality/quantity of cerumen were recorded, and the clinical ear scores were calculated as shown in Table 2. Each score was assessed for left and right ears and then the sum was calculated.

2.5. Bacterial and fungal analyses

Ear swab samples were cultured onto Sabouraud dextrose agar medium with 0.5 % chloramphenicol and on Dixon agar and incubated at 32 °C for 10 days for yeast isolation, whereas on Columbia blood agar (CBA), Mannitol Salt Agar (MSA) and MacConkey agar (MCK),

Table 2

Evaluation scales employed for the clinical signs.

Grade	Signs of Pruritus	Presence of Cerumen
1	Absent	Absent
2	Mild without external skin alteration	Clear with low volume
3	Moderate with mild skin alterations of the external ear	Brown cerumen with medium sized volume
4	Severe with marked skin alterations of the external ear	Dark cerumen, large volume

incubated at 35 °C for 48 h in aerobic conditions, for bacteria isolation. The colonies were counted and expressed as colony-forming units per sample (CFU). For each sample positive to yeasts (>20 CFU), the colonies were examined microscopically after Gram staining and identified by microscopical features (e.g., urea hydrolysis, the ability to assimilate Tween, growth on PEG-35 castor oil, and β -glucosidase activity), and sugar assimilation system (Cafarchia et al., 2011). For bacteria after a Gram staining, the colonies were biochemically identified by means of API-system (Biomérieux, Marcyilly-le-Châtel, France) (Lyskova et al., 2007).

2.6. Ear flushing

A quantitative assessment of live (motile) ear mites by ear-duct flushing (Fig. 1), mite collection and live mite count was performed on $D42 \pm 2$ on all dogs. Clinical alterations were evaluated prior to ear flushing to ensure that the clinical presentation was unbiased.

The ear-ducts were flushed with an appropriate ear cleaning solution (i.e., 5% aqueous solution of docusate sodium) and massaged lightly externally until obtaining a sufficient melting of the ear-duct content. The melted solution was collected and drained into a 38 μ m sieve, followed by 2 mL of a warm saline solution flush of the ear duct into the same sieve and collected into a 50 mL labelled tube, separately for each ear. Then, both ears were otoscopically examined and the flushing procedure was repeated until both ear-ducts were judged clean, thus, without persistent cerumen deposits and/or mites observed. The contents of both tubes were immediately examined and stored in labelled 1.5 mL tubes. All collected material was observed on the same day at the stereomicroscope, and all the live mites (adult and immature) were morphologically identified (Sweatman, 1958), counted and recorded for each ear.

2.7. Assessment of efficacy and Statistical analyses

The primary criterion for the assessment of the efficacy was the live (motile) ear mite counts in animals on D42. The study was considered valid if at least 75 % of dogs in the negative Control Group were still positive for live (motile) *O. cynotis* in at least one ear. Arithmetic mean for each group was calculated. Efficacy for the treated group was determined by calculating the percent efficacy as $100 \times [(C-T)/C]$, where C is the mean among untreated controls and T is the mean among the treated animals. The counts of the treated group were compared to the counts of the untreated control group at each time point using a non-parametric Wilcoxon sum rank test. The two groups listed as a fixed effect, and the allocation blocks listed as a random effect. All testing were two-sided at the significance level $\alpha = 0.05$. The number of mite-free dogs were compared using a Fisher exact test. The secondary criterion for the assessment of the efficacy were the clinical lesion



Fig. 1. In detail, the ear flushing procedure in a dog of the untreated control group at the D42 post-enrolment.

scores, the semi-quantitative ear mite counts, and the assessment of bacterial and fungal populations (i.e., D0, D30, D42). Data regarding ear mite, bacterial and fungal infections at each time point were recorded in a Microsoft Excel spreadsheet. The groups were compared using non-parametric (Kruskal Wallis test) and two-sided with continuity correction (Wilcoxon sum rank test) tests through “R”. The success rate was determined for each time point as the percentage of dogs in the treated group that showed lower clinical scores versus the pre-treatment and a clinical score of “1” or “2”. Results of bacteriological and mycological examination were processed using chi-squared test, with significance specified at 0.05.

3. Results

3.1. Antiparasitic efficacy

At D0, the total count of ear mites for each dog, ranged from 1 to 21 and 1 to 24 for the control group and treated group, respectively, while at D42, all treated dogs became negative, whereas the untreated ones remained positive, except for two.

Based on arithmetic means, an average of 4.1 live mites (adults and immatures) per control dog, and an average of 0 live mites per afoxolaner-treated dogs, was recorded after ear flushing (Table 4). Based on the ear-mite counts, no live mites were present in the afoxolaner-treated group compared to the control group at D42 ($p = 0.00073$), the significant difference between the two group was obtained using the Wilcoxon rank sum test. The antiparasitic efficacy of the tested product against *O. cynotis* was 100 %. The comparison of mite free dogs between D0 and D42 was significant ($p = 0.00071$), using the Fisher’s Exact Test.

3.2. Clinical examinations and health observations

None of the clinical signs associated with the mite infestations was observed during the clinical examinations, except for five cases at D0, two dogs in the control group and three dogs in the treated group with sum scores >2. The veterinarian carefully examined and judged all enrolled dogs to be fit to continue at each assessment throughout the study (D0, D30 and D42). No serious health issues were recorded during the study, with only mild signs consistent with *O. cynotis* infestation observed. No adverse events related to the administration of afoxolaner were observed.

The clinical scores did not significantly change within the control group during the whole study period ($p = 0.24$), whereas they improved significantly in the treated group: $p = 9.798 \times 10^{-7}$ and $p = 5.47 \times 10^{-5}$ (D0/D30 score) with Kruskal-Wallis and Wilcoxon rank sum tests, respectively (Table 3).

3.3. Bacterial and fungal assessments

Dogs in control and treated groups showed a positivity at D0 for *Malassezia pachydermatis* ($n = 12$; 60 %) that decreased to ($n = 7$, 35 %) after the whole period of study. In particular, 4 dogs of the treated group were positive for *M. pachydermatis* at D0. Of these, 3 dogs became negative at D42, while out of 8 positive dogs for *M. pachydermatis* in the control group, only 2 became negative at D42. The difference in dogs becoming negative at the end of the study for the fungal infection is not statistically significant between the two groups (OR = 0.11; $\chi^2 = 2.74$; $p = 0.097$).

Dogs were also positive to bacterial infections due to *Bacillus* spp. ($n = 3$; 15 %), *Staphylococcus sciuri* ($n = 1$; 5%), *Staphylococcus chromogenes* ($n = 3$; 15 %), *Staphylococcus lentus* ($n = 1$; 5%), *Staphylococcus pseudointermedius* ($n = 1$; 5%); *Proteus* spp. ($n = 3$; 15 %); *Staphylococcus* spp. ($n = 1$; 5%). One dog was also coinfecting by *S. chromogenes* and *S. sciuri*.

Table 3

Evolution of the clinical ear scores from D0 to D42 in both untreated control group and afoxolaner-treated group (2.5 mg/kg on Days 0 and 30).

	D0	D30	D42
Untreated control group	2	2	6
	1	2	2
	1	4	4
	1	2	0
	2	1	0
	5	3	2
	1	2	0
	1	2	2
	2	2	1
	1	2	4
Mean	1.7	2.2	2.1
Afoxolaner-treated group	2	0	0
	1	0	0
	5	0	0
	3	0	0
	2	0	0
	1	0	0
	4	0	0
	2	0	0
	2	0	0
	2	0	0
	2	0	0
	Mean	2.4	0

Table 4

Evolution of the ear mite counts from D0 to D42 in both untreated control group and afoxolaner-treated group (2.5 mg/kg on Days 0 and 30). The ear mite counts were performed by deep swab method at D0 and D30, whereas ear flushing for the final count at D42.

	D0	D30	D42	
Untreated control group	2	6	11	
	1	2	0	
	3	9	9	
	2	5	1	
	2	1	1	
	21	7	8	
	2	3	0	
	1	5	1	
	1	4	4	
	4	4	6	
	Mean	3.9	4.6	4.1
	Afoxolaner-treated group	2	0	0
1		0	0	
18		0	0	
10		0	0	
4		0	0	
1		0	0	
24		0	0	
6		0	0	
3		0	0	
5		0	0	
Mean		7.4	0	0

4. Discussion

The administration of two oral monthly doses of afoxolaner, at minimum dose of 2.5 mg/kg, was effective in rapidly eliminating ear mites, which resulted in 100 % of antiparasitic efficacy against *O. cynotis* infestations, as assessed 42 days after the first administration.

This efficacy was higher than that observed in the study published by Carithers et al. (2016; 98 % efficacy based on mite count with 2 out of 8 dogs still infested at D28), although the dogs in that trial were infested by a higher number of ear mites and treated just once with afoxolaner. On the other hand, our partial results at D30, before the second administration of the systemic insecticide, showed that all the treated dogs were negative for the presence of ear mites at the otoscopic

examination and deep swab method. The high efficacy of afoxolaner against ear mites, herein described, may be explained by the number of doses administered and the difference in time between the last product administration (D30) and the final ear mite count (D42), compared to the aforementioned study. Indeed, in another similar trial, in which the difference in efficacy of two different treatments against the ear mite infestation (i.e., 10 % imidacloprid + 2.5 % moxidectin and selamectin) was evaluated, there was an increase in the mite clearance rate from the final administration of the products (i.e., up to 71 % at D28) to the last ear mite count evaluation (i.e., up to 82 % at D56) (Arther et al., 2015). Therefore, the treatment efficiency improves the ear mite clearance rate in the infested dogs gradually after administration. The acaricidal efficacy of afoxolaner has also been observed for other mite species infesting dogs, namely *Sarcoptes scabiei* (i.e., 100 % efficacy; Beugnet et al., 2016a) and *Demodex* spp. (i.e., 99.2 % efficacy after the second dose at D28; Beugnet et al., 2016b). This study confirmed the wide applicability of this systemic insecticide in the treatment of different dog mange infestations.

The clinical scores showed a low number of dogs with marked signs at D0. However, the low mite count intensity in the dogs may explain this lack of clinical severity. Nevertheless, the treatment with afoxolaner induced a significant improvement with disappearance of any signs at D30. This result demonstrates that the parasitological cure from the ear mange infestation may be positively related to the resolution of its clinical signs, as shown also in a previous study (Becskei et al., 2018). Furthermore, *Otodectes* infestation is usually accompanied by bacterial and fungal infections (Nardoni et al., 2014). In the present study, although a decrease in the number of dogs positive for *M. pachydermatis* infection was observed at D42 in the treated group, no statistically significant difference was found in the treatment effect on the yeast infection between the two groups. This could be related to the limited sample of dogs infected with *Malassezia* spp.. Further analysis should be conducted to assess the impact of ear mite treatment to the ear microbiote recovery as suggested in a previous work (Tarallo et al., 2009). Most of the cases of bacterial infections were due to *Staphylococcus* spp. and *Bacillus* spp., which are well-known to be opportunistic pathogens and normal contaminants of the skin (Nardoni et al., 2014). In addition, in three cases (i.e., one dog of G2 and two dogs of G1), a specific treatment of *Proteus* spp. infection was advocated considering the non-opportunistic role of this pathogen (Lyskova et al., 2007). However, all the above-mentioned bacteria have already been associated to the ear infections in dogs (Nardoni et al., 2014).

5. Conclusion

The high efficacy achieved with NexGard® (afoxolaner) under natural field conditions confirms the results of experimental ear mite infestation. It confirms the possibility to use isoxazolines to treat ear mange in dogs, as it has been recently demonstrated in cats for NexGard Combo® (Tielemans et al., 2021).

Ethical standards

Animals were handled with regard for their well-being in compliance with the relevant BIAH Animal Care and Use/Ethics Committee approvals and following the approval of the Ethical Committee of the Department of Veterinary Medicine of the University of Bari, Italy (Prot. Uniba 07/2021). Dog owners agreed with all the procedures and signed the informed consent before participating in the study.

Financial support

This work was supported by Boehringer Ingelheim Animal Health (France, Europe). The funders contributed to the data analyses and preparation of the manuscript.

CRedit authorship contribution statement

Rossella Panarese: Conceptualization, Methodology, Project administration, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Roberta Iatta:** Conceptualization, Data curation, Project administration, Writing - review & editing. **Riccardo Paolo Lia:** Methodology, Investigation, Writing - review & editing. **Wilfried Lebon:** Conceptualization, Methodology, Validation, Resources. **Frederic Beugnet:** Conceptualization, Validation, Resources, Formal analysis, Writing - review & editing. **Domenico Otranto:** Conceptualization, Supervision, Data curation, Writing - review & editing.

Declaration of Competing Interest

Frederic Beugnet and Wilfried Lebon are employed by the commercial company Boehringer Ingelheim Animal Health (France, Europe). The remaining authors have declared that no competing interests exist.

Acknowledgements

Authors would like to thank Salvatore Luciano (Veterinary practitioner, University of Naples) and Paolo Greco (student attending the Animal Science Course, University of Bari) for their support during the sample collection and field activities.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetpar.2021.109607>.

References

- Arther, R.G., Davis, W.L., Jacobsen, J.A., Lewis, V.A., Settje, T.L., 2015. Clinical evaluation of the safety and efficacy of 10% imidacloprid + 2.5% moxidectin topical solution for the treatment of ear mite (*Otodectes cynotis*) infestations in dogs. *Vet. Parasitol.* 210, 64–68.
- Baker, A.S., 1999. Mites and Ticks of Domestic Animals – an Identification Guide and Information Source. NHM, London, UK, pp. 89–91.
- Becskei, C., Cuppens, O., Mahabir, S.P., 2018. Efficacy and safety of sarolaner in the treatment of canine ear mite infestation caused by *Otodectes cynotis*: a non-inferiority study. *Vet. Dermatol.* 29, 100–e39.
- Beugnet, F., Bouhsira, É., Halos, L., Franc, M., 2014. Preventive efficacy of a topical combination of fipronil-(S)-methoprene-eprinomectin-praziquantel against ear mite (*Otodectes cynotis*) infestation of cats through a natural infestation model. *Parasite* 21.
- Beugnet, F., de Vos, C., Liebenberg, J., Halos, L., Larsen, D., Fourie, J., 2016a. Efficacy of afoxolaner in a clinical field study in dogs naturally infested with *Sarcoptes scabiei*. *Parasite* 23, 26.
- Beugnet, F., Halos, L., Larsen, D., de Vos, C., 2016b. Efficacy of oral afoxolaner for the treatment of canine generalised demodicosis. *Parasite* 23.
- Cafarchia, C., Gasser, R.B., Figueredo, L.A., Latrofa, M.S., Otranto, D., 2011. Advances in the identification of *Malassezia*. *Mol. Cell. Probes* 25 (1), 1–7.
- Carithers, D., Crawford, J., de Vos, C., Lotriet, A., Fourie, J., 2016. Assessment of afoxolaner efficacy against *Otodectes cynotis* infestations of dogs. *Parasite Vectors* 9 (1), 1–5.
- Curtis, C.F., 2004. Current trends in the treatment of *Sarcoptes*, *Cheyletiella* and *Otodectes* mite infestations in dogs and cats. *Vet. Dermatol.* 15, 108–114.
- Halos, L., Lebon, W., Chalvet-Monfray, K., Larsen, D., Beugnet, F., 2014. Immediate efficacy and persistent speed of kill of a novel oral formulation of afoxolaner (NexGard®) against induced infestations with *Ixodes ricinus* ticks. *Parasite Vectors* 7, 452.
- Hampel, V., Knaus, M., Schäfer, J., Beugnet, F., Rehbein, S., 2018. Treatment of canine sarcoptic mange with afoxolaner (NexGard®) and afoxolaner plus milbemycin oxime (NexGard Spectra®) chewable tablets: efficacy under field conditions in Portugal and Germany. *Parasite* 25, 63.
- Kraft, W., Kraiss-Gothe, A., Gothe, R., 1988. *Otodectes cynotis* infestation of dogs and cats: the biology of the agent, epidemiology, pathogenesis and diagnosis and case description of generalized mange in dogs. *Tierarztl. Prax.* 16, 409–415.
- Lebon, W., Beccati, M., Bourdeau, P., Brement, T., Bruet, V., Cekiera, A., Crosaz, O., Darmon, C., Guillot, J., Mosca, M., Pin, D., Popiel, J., Handwerker, D.P., Larsen, D., Tielemans, E., Beugnet, F., Halos, L., 2018. Efficacy of two formulations of afoxolaner (NexGard® and NexGard Spectra®) for the treatment of generalised demodicosis in dogs, in veterinary dermatology referral centers in Europe. *Parasite Vectors* 11, 506.
- Lyskova, P., Vydralova, M., Mazurova, J., 2007. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. *J. Vet. Med. Series A* 54 (10), 559–563.
- Mullen, G., O'connor, B., 2002. Mites (Acari). In: Mullen, G., Durden, L. (Eds.), *Med Vet Entomol.* Academic, UK, pp. 449–516.
- Nardoni, S., Ebani, V.V., Fratini, F., Mannella, R., Pinferi, G., Mancianti, F., Finotello, R., Perrucci, S., 2014. *Malassezia*, mites and bacteria in the external ear canal of dogs and cats with otitis externa. *Slov. Vet. Res.* 51 (3), 113–118.
- Otranto, D., Milillo, P., Mesto, P., De Caprariis, D., Perrucci, S., Capelli, G., 2004. *Otodectes cynotis* (Acari: psoroptidae): examination of survival off-the-host under natural and laboratory conditions. *Exp. Appl. Acarol.* 32 (3), 171–180.
- Pappas, J.C., Kartz, T.L., 1995. Evaluation of a treatment for the ear mite, *Otodectes cynotis*, in kittens. *Feline Prac.* 23, 21–24.
- Scherk-Nixon, M., Baker, B., Pauling, G.E., Hare, J.E., 1997. Treatment of feline otocariasis with 2 otic preparations not containing miticidal active ingredients. *Can. Vet. J.* 38, 229–230.
- Scott, D.W., Horn, J.R.T., 1987. Zoonotic dermatoses of dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 17, 117–144.
- Shanks, D.J., McTier, T.L., Rowan, T.G., Watson, P., Thomas, C.A., Bowman, D.D., Hair, J.A., Pengo, G., Genchi, C., Smothers, C.D., Smith, D.G., Jernigan, A.D., 2000. The efficacy of selamectin in the treatment of naturally acquired aural infestations of *Otodectes cynotis* on dogs and cats. *Vet. Parasitol.* 91, 283–290.
- Shoop, W.L., Hartline, E.J., Gould, B.R., Waddell, M.E., McDowell, R.G., Kinney, J.B., Lahm, G.P., Long, J.K., Xu, M., Wagerle, T., Jones, G.S., Dietrich, R.F., Cordova, D., Schroeder, M.E., Rhoades, D.F., Benner, E.A., Confalone, P.N., 2014. Discovery and mode of action of afoxolaner, a new isoxazoline parasiticide for dogs. *Vet. Parasitol.* 201, 179–189.
- Sweatman, G.K., 1958. Biology of *Otodectes cynotis*, the ear canker mite of carnivores. *Can. J. Zool.* 36, 849–862.
- Taenzler, J., De Vos, C., Roepke, R.K., Frénais, R., Heckerth, A.R., 2017. Efficacy of fluralaner against *Otodectes cynotis* infestations in dogs and cats. *Parasite Vectors* 10 (1), 1–6.
- Tarallo, V.D., Lia, R.P., Sasanelli, M., Cafarchia, C., Otranto, D., 2009. Efficacy of Amitraz plus Metaflumizone for the treatment of canine demodicosis associated with *Malassezia pachydermatis*. *Parasite Vectors* 2 (1), 1–5.
- Tielemans, E., Prullage, J., Tomoko, O., Liebenberg, J., Capári, B., Sotiraki, S., Kostopoulou, D., Ligda, P., Ulrich, M., Knaus, M., 2021. Efficacy of a novel topical combination of esafloxolaner, eprinomectin and praziquantel against ear mite (*Otodectes cynotis*) infestations in cats. *Parasite* 28, 26.