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Evaluation of the efficacy of afoxolaner against two European dog tick species: *Dermacentor reticulatus* and *Ixodes ricinus*

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ABSTRACT

The acaricidal efficacy of a novel oral formulation of afoxolaner (NEXGARD®, Merial) against two European tick species was assessed in dogs experimentally infested with *Ixodes ricinus* and *Dermacentor reticulatus*. Three studies, each characterized by a negative controlled randomized block design, were conducted with a total of 52 beagle or mongrel dogs of both sexes. Starting 2 days before treatment, each dog was infested weekly with approximately 50 ticks. The number of live ticks was counted at 48 h post-treatment (Day 2) as well as 48 h following each infestation on Days 9, 16, 23, and 30. Afoxolaner, administered at an average dose of 2.7 mg/kg bodyweight (range 2.5–2.9 mg/kg), rapidly eliminated the pre-existing tick infestations with over 99% acaricidal efficacy and controlled the weekly re-infestations for up to 30 days post treatment with over 96% efficacy on both tick species. Afoxolaner provides excellent acaricidal efficacy against these two major European tick species using the oral route of administration.

1. Introduction

Ticks are endemic throughout Europe with more than twelve different species, which have varying biology and geographic distribution (Chomel, 2011). *Ixodes ricinus* and *Dermacentor reticulatus* commonly infest dogs and are vectors of various canine vector borne pathogens including *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, both transmitted by *I. ricinus*, and *Babesia canis* transmitted by *D. reticulatus* (Chomel, 2011; Dantas-Torres et al., 2012). *I. ricinus* is found throughout Europe, except on the Mediterranean border, and *D. reticulatus* is widely distributed with areas of tick concentration dependent upon local environmental conditions (Beugnet and Chalvet-Monfray, 2013).

The distribution of both tick species has been expanding in Europe as a result of climate change (Beugnet and Chalvet-Monfray, 2013; Daniel et al., 2003; Gray et al., 2009; Smith et al., 2011). The period of tick activity also is increasing in Europe. For example, in Hungary the total duration of *I. ricinus* activity was shown to be 2 months longer in 2002 compared to the duration in 1950, and ticks were also found in winter, a finding not described in 1950 (Smith et al., 2011). Similar findings are described for *I. ricinus* or *D. reticulatus* in Belgium, Switzerland, Poland, Germany, Slovenia and Slovakia (Bullova et al., 2009; Chomel, 2011; Reis et al., 2011; Siroky et al., 2011). Although more common in colder climates, *I. ricinus* can be found in the warmer climate conditions of the Mediterranean region as demonstrated

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Table 1
Study design to evaluate the efficacy of afoxolaner against *Dermacentor reticulatus* and *Ixodes ricinus*.

Tick species	Study code	Dog breed, age (months), body weight (kg)	Number of dogs in control/treated groups	Average dosage of afoxolaner mg/kg (min–max)
Dermacentor reticulatus	A – unfed dogs	Cross breed, >6, 11.0–18.4	8/8	2.71 (2.5–2.9)
	B – fed dogs	Cross breed, >6, 9.6–18.1	10/10	2.76 (2.5–2.9)
Ixodes ricinus	C – fed dogs	Beagle, 11–13, 7.9–12.5	8/8	2.73 (2.5–2.95)

by Dantas-Torres and Otranto (2013), who showed that *I. ricinus* is present in southern Italy where it remains active throughout the year with spatiotemporal distribution patterns that are distinct from central and north European populations. The expansion of the geographic and temporal incidence of ticks throughout Europe and the increased movement of people and their companion animals between countries strengthen the need for effective tick control measures for dogs year-round (Otranto et al., 2009a,b).

The present study describes the results of three experimental studies that assessed the efficacy of afoxolaner, a new insecticide–acaricide administered orally in a soft chewable formulation (Nexgard®, Merial), against *I. ricinus* and *D. reticulatus* in dogs. This new acaricide was tested for its curative property (i.e., its ability to kill ticks when administered to an infested dog), and for its prophylactic properties (i.e., its ability to prevent tick re-infestations for 30 days after treatment).

2. Materials and methods

Fifty-two dogs were included in three laboratory studies. Each study included 16–20 beagle or mongrel dogs of both sexes using a negative controlled randomized block design (Table 1). All dogs were healthy, >6 months of age, between 7.9 and 18.4 kg bodyweight at inclusion, and no dogs had been infested by ticks nor treated with any insecticidal–acaricidal drug in a 3-month period before inclusion.

The health condition of all dogs was monitored at least once daily and additionally once per hour during the first four h post treatment. They were acclimated to the study conditions for at least 7 days prior to treatment. All dogs had free access to water and were fed a commercial diet provided in an amount and manner that supplied nutrient and energy requirements to ensure their health and well-being. All animal procedures in this study were reviewed and approved by the Merial Ethics Committee (USDA, 2008). The study design was in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Marchiondo et al., 2013), and was conducted in accordance with Good Clinical Practices as described in *International Cooperation on Harmonisation* of Technical Requirements for Registration of *Veterinary* Medicinal Products (VICH) guideline GL9 (EMEA, 2000).

In each of the 3 studies, two groups of equal size were randomly formed based on decreasing pre-treatment tick counts (performed during acclimation, 48 h after infestation). Dogs were either untreated or treated once orally with the appropriate combination of soft chews containing afoxolaner (day of treatment was Day 0). Four sizes of chews were available: 0.5 g, 1.25 g, 3 g and 6 g, containing respectively 11.3 mg, 28.3 mg, 68 mg and 136 mg of afoxolaner. As the soft chews cannot be cut, the dosing was administered by giving one or more of the chews to be as close as possible to the minimum effective dose of 2.5 mg/kg, resulting in an average dose of 2.7 mg/kg of afoxolaner per each treated group (Table 1). Dogs were fasted overnight prior to treatment and fed approximately four h after treatment in Study A but were fed in the hour before treatment in Studies B and C.

On Days −2, 7, 14, 21 and 28, all dogs were infested with 50 unfed adult ticks (with approximately equal sex ratio for *D. reticulatus* in Studies A and B, and 50 females of *I. ricinus*, with 10 additional males to stimulate attachment, in Study C). Ticks were from laboratory-maintained populations that had been established from ticks collected in field locations in Europe. Live ticks were counted and removed 48 h after treatment or infestations on Days 2, 9, 16, 23, and 30. These counts consisted of the methodical examination of all body areas using finger tips and/or a coarse tooth comb to sort through the hair and locate all ticks on the animal, as described by Marchiondo et al. (2013). All personnel conducting tick counts and health observations were blinded to treatment groups.

For each tick count, including male and female ticks, the total count of live adult ticks was transformed to the natural logarithm of (count + 1) to calculate the geometric mean for each treatment group. The percent reduction of the tick counts from treated dogs compared to those from untreated dogs (= percentage efficacy) was calculated using the formula $[(C - T)/C] \times 100$, where C is the geometric mean for the control group and T is the geometric mean for the treated group at the same time point. The tick counts of the treated and untreated groups were also compared using Friedman's test. All testing was two-sided at the significance level $p = 0.05$.

For each study, the therapeutic efficacy of afoxolaner against Day −2 infestation and the efficacy of afoxolaner in the prevention of weekly tick re-infestations starting on Day 7 after treatment were assessed.

3. Results

In the control groups, the geometric mean of live ticks counted at each time point was between 19.9 and 32.3 for infestations with *D. reticulatus* and between 23.4 and 34.2 for *I. ricinus* (Table 2). To allow the comparison between control and treated groups, the minimum retention rate of ticks should be at least 20% (i.e., 10 ticks for an

Table 2
Geometric mean number of live ticks in the control group[a] (min–max tick count) 48 h after infestation.

Tick species	Study code	Study day				
		2	9	16	23	30
Dermacentor reticulatus	A	30.6 (14–44)	21.5 (13–33)	25.7 (14–40)	29.4 (15–43)	19.9 (9–32)
	B	32.3 (17–48)	25.2 (8–47)	24.6 (13–39)	22.1 (13–48)	20.5 (18–43)
Ixodes ricinus	C	34.2 (25–42)	27.0 (20–37)	23.6 (15–28)	23.4 (19–28)	23.8 (19–32)

[a] 50 unfed ticks of equal sex ratio were used for infestation with *D. reticulatus*. 50 unfed female ticks with approximately 10 males were used for infestation with *I. ricinus*.

Table 3
Percent efficacy of afoxolaner against *Dermacentor reticulatus* and *Ixodes ricinus* and 48 h after treatment/infestation (geometric means count in the treated group).[a]

Tick species	Study code	Percent efficacy				
		Study day				
		2[b]	9[c]	16[c]	23[c]	30[c]
Dermacentor reticulatus	A – unfed dogs	100 (0.0)	98.5 (0.3)	97.9 (0.5)	98.2 (0.5)	97.6 (0.5)
	B – fed dogs	100 (0.0)	99.7 (0.1)	99.2 (0.2)	98.0 (0.4)	96.4 (0.7)
Ixodes ricinus	C – fed dogs	100 (0.0)	100. (0.0)	100 (0.0)	100 (0.0)	99.6 (0.1)

[a] A significant difference ($p < 0.002$) was demonstrated between the geometric mean counts of the control and treated group in each study at each time point.
[b] Therapeutic efficacy of afoxolaner against Day −2 infestation.
[c] Prophylactic efficacy against weekly tick re-infestations.

infestation with 50) in order to make a valid assessment of tick efficacy (Marchiondo et al., 2013). The control dogs were adequately infested in the 3 studies.

In the treated groups, the tick reduction was significant ($p < 0.002$) compared to the control groups for each time point of each study (Table 3). Afoxolaner treatment provided a complete elimination of all ticks when infestation existed prior to treatment (therapeutic efficacy) and provided over 96.4% acaricidal efficacy against *D. reticulatus* and over 99.6% against *I. ricinus* up to Day 30 (Table 3) when infestations occurred after treatment (prophylactic efficacy). No adverse effects related to treatment were observed.

4. Discussion

A single oral treatment with the chewable formulation of afoxolaner achieved 100% therapeutic efficacy for treating pre-existing infestations by *I. ricinus* and *D. reticulatus*. It also controlled re-infestations of ticks within 48 h for 4 weeks after treatment as demonstrated by the prophylactic efficacy, which was over 96.4% for the 2 studies against *D. reticulatus* and over 99.6% for the *I. ricinus* study. No difference was observed in efficacy against *D. reticulatus* ticks between fasted (Study A) and fed (Study B) dogs. Treated dogs in all three studies accepted the afoxolaner chews without adverse reactions, based on hourly post-treatment observations and daily observations.

The acaricidal efficacy of afoxolaner against *I. ricinus* and *D. reticulatus* observed in these studies was similar to what is usually observed with topical products. For example, against *I. ricinus*, two spot-on solutions, one with pyriprole and one with permethrin/imidacloprid provided a curative efficacy of 100% and 67.0%, respectively on Day 2, and greater than 98.7% prophylactic efficacy up to 30 days when assessed 48 h after tick infestations (Epe et al., 2003; Schuele et al., 2008). In a study comparing three topical treatments against *D. reticulatus* (Tielemans et al., 2010), the efficacies were 100%, 76% and 70% on Day 30 for fipronil/(S)-methoprene, permethrin/imidacloprid and metaflumizone/amitraz, respectively. The oral formulation of afoxolaner is the first one to provide an efficacy against tick for a month, as it is described for topical products. Nevertheless, no direct comparison is available.

The systemic distribution of the tested product offer advantages compared to the topical formulations. One benefit is the lower possibility of exposure of the owner during the time necessary for a topical product to be absorbed through the skin of a treated dog. Another significant advantage of an orally administered, systemically active product is that rainy conditions, shampooing, or other concurrent topical treatments will not interfere with the efficacy (Beugnet and Franc, 2012).

In conclusion, the chewable formulation containing the new insecticide–acaricide afoxolaner is a convenient and efficacious ectoparasiticide treatment for dogs that treats and prevents tick infestations for up to 1 month.

Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. The authors are current employees or contractors of Merial.

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References

Beugnet, F., Franc, M., 2012. Insecticide and acaricide molecules and/or combinations to prevent pet infestation by ectoparasites. Trends Parasitol. 28, 267–279.

Beugnet, F., Chalvet-Monfray, K., 2013. Impact of climate change in the epidemiology of vector-borne diseases in domestic carnivores. CIMID 36, 559–566.

Bullova, E., Luka, M., Stanko, M., Pet'ko, B., 2009. Spatial distribution of *Dermacentor reticulatus* tick in Slovakia in the beginning of the 21st century. Vet. Parasitol. 165, 357–360.

Chomel, B., 2011. Tick-borne infections in dogs—an emerging infectious threat. Vet. Parasitol. 179, 294–301.

Daniel, M., Danielová, V., Kríz, B., Jirsa, A., Nozicka, J., 2003. Shift of the tick *Ixodes ricinus* and tick-borne encephalitis to higher altitudes in Central Europe. J. Clin. Microbiol. Infect. Dis. 22 (5), 327–328.

Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 28 (10), 437–446.

Dantas-Torres, F., Otranto, D., 2013. Seasonal dynamics of *Ixodes ricinus* on ground level and higher vegetation in a preserved wooded area in southern Europe. Vet. Parasitol. 192, 253–258.

EMEA, 2000. Guideline on Good Clinical Practices. VICH Topic GL9 (GCP). EMWA/CVMP/VICH/595/98-Final, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_g uideline/2009/10/WC500004343.pdf

Epe, C., Coati, N., Stanneck, D., 2003. Efficacy of the compound preparation imidacloprid 10% (w/v)/permethrin 50% (w/v) spot on against ticks (*I. ricinus*, *R. sanguineus*) and fleas (*C. felis*) on dogs. Parasitol. Res. 90 (Suppl. 3), S122–S124.

Gray, J.S., Dautel, H., Estrada-Pena, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis., 12, Article ID 593232.

Marchiondo, A.A., Holdsworth, P.A., Fourie, L.J., Rugg, D., Hellmann, K.E., Snyder, D.E., Dryden, M.W., 2013. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet. Parasitol. 194, 84–97.

Otranto, D., Dantas-Torres, F., Breitschwerdt, E.B., 2009a. Managing canine vector-borne diseases of zoonotic concern: part one. Trends Parasitol. 25, 157–163.

Otranto, D., Dantas-Torres, F., Breitschwerdt, E.B., 2009b. Managing canine vector-borne diseases of zoonotic concern: part two. Trends Parasitol. 25, 228–235.

Reis, C., Cote, M., Paul, R.E.L., Bonnet, S., 2011. Questing ticks in suburban forest are infected. Vector Borne Zoonotic Dis. 11, 907–916.

Schuele, G., Barnett, S., Bapst, B., Cavaliero, T., Luempert, L., Strehlau, G., Young, D.R., Moran, C., Junquera, P., 2008. Efficacy of a single application of a 12.5% pyriprole topical solution against laboratory infestations with ticks (*Ixodes ricinus Dermacentor reticulatus* and *Rhipicephalus sanguineus*) on dogs. Vet. Parasitol. 151, 311–317.

Siroky, P., Kubelová, M., Bednář, M., Modrý, D., Hubálek, Z., Tkadlec, E., 2011. The distribution and spreading pattern of *Dermacentor reticulatus* over its threshold area in the Czech Republic—how much is range of this vector expanding? Vet. Parasitol. 183, 130–135.

Smith, F.D., Ballantyne, R., Morgan, E.R., Wall, R., 2011. Prevalence, distribution and risk associated with tick infestation of dogs in Great Britain. Med. Vet. Entomol., http://dx.doi.org/10.1111/j.1365-2915.2011.00954.x.

Tielemans, E., Manavella, C., Pollmeier, M., Chester, T., Murphy, M., Gale, B., 2010. Comparative acaricidal efficacy of the topically applied combinations fipronil/(S)methoprene, permethrin/imidacloprid and metaflumizone/amitraz against *Dermacentor reticulatus*, the European dog tick (ornate dog tick, Fabricius, 1794) in dogs. Parasite 17, 343–348.

USDA, 2008. Animal Welfare Regulations, 9CFR, http://awic.nal.usda.gov/government-and-professional-resources/federal-laws/animal-welfare-act