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Assessment of the efficacy of orally administered afoxolaner against *Rhipicephalus sanguineus* sensu lato

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ABSTRACT

Two studies were conducted to confirm that a single oral dose of the novel insecticide/acaricide afoxolaner is efficacious against existing infestations of *Rhipicephalus sanguineus* sensu lato in dogs and can control re-infestation for up to 35 days. Each study utilized 16 purpose bred adult dogs using a controlled randomized block design. One or two days prior to treatment, all dogs were infested with 50 unfed adult ticks. On Day 0 one group was treated with an oral chewable formulation of afoxolaner at a dose as close as possible to the minimum dose of 2.5 mg/kg. Weekly re-infestations with 50 adult unfed ticks were repeated for five weeks. Forty-eight hours after treatment and after each re-infestation, the number of remaining live ticks on each dog was counted. Treatment with afoxolaner resulted in efficacies of 98.8–100% within 48 h on existing tick infestations, while the efficacy against new tick infestations was >95.7% over five weeks.

1. Introduction

Ripicephalus sanguineus sensu lato, the brown dog tick, most probably originated in Africa, but currently has a world-wide distribution and is an important parasite of dogs and other domestic animals (Dantas-Torres, 2010). This tick species is an important vector of a diverse range of pathogens, such as *Babesia*, *Cercopithifilaria*, *Hepatozon*, *Ehrlichia*, and *Rickettsia* (Dantas-Torres, 2010; Gray et al., 2013). Control of tick infestations can be a primary means of preventing these infections (Beugnet and Franc, 2012; Otranto et al., 2009a,b) and limiting other adverse consequences of tick infestations, such as flaccid paralysis (Blagburn and Dryden, 2009; Otranto et al., 2012). This article describes a series of 2 studies that were performed to demonstrate the efficacy of afoxolaner, a novel insecticide–acaricide, in an oral chewable formulation (Nexgard®, Merial) against *R. sanguineus*.

2. Materials and methods

2.1. Experimental design

Two studies were conducted to assess the efficacy of afoxolaner against *R. sanguineus* sensu lato using two tick strains from different parts of the world. Study A was performed in South Africa, and Study B in Australia. All animal procedures in this study were reviewed and approved by the Merial Institutional Animal Care and Use Committee (USDA, 2008). The design of the studies 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 (EMEA, 2000).

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Table 1
Study design to assess the efficacy of afoxolaner against *Rhipicephalus sanguineus* sensu lato.

Study and study location	Dog's sex	Body weight (kg)	Oral treatment	Number of ticks for each infestation	Tick infestation days	Tick count days
A. South Africa	7 Males, 9 females	11.0–18.4	Day 0	50	−2, 7, 14, 21, 28, 35	2, 9, 16, 23, 30, 37
B. Australia	7 Males, 9 females	6.0–30.8	Day 0	50	−1, 7, 14, 21, 28, 35	2, 9, 16, 23, 30, 37

Sixteen mixed breed dogs were included in Study A, and 4 each of Beagles, Labrador Retrievers, German Shorthaired Pointers, and Jack Russell Terriers in Study B. All studies followed a controlled, randomized, block design (Table 1). All dogs were given a physical examination prior to allocation to study groups and confirmed to be healthy. They were not infested by ticks and did not receive any ectoparasiticide treatment within the previous 3 months. A pre-treatment tick infestation was conducted in each study and used for allocation. For each study, 16 dogs were placed in 8 blocks of 2 dogs based on descending tick counts. Within each block, each dog was randomly assigned to either the control or the afoxolaner-treated group. Each dog was housed individually. Daily health observations were made throughout each study and the presence or absence of any health issue or adverse experience was documented. In addition, hourly health observations were made for 4 h following treatment on Day 0.

Each study used unfed adult ticks from laboratory-maintained populations. No tick strains were known to be resistant to any ectoparasiticide.

2.2. Experimental procedures

On Day 0, dogs in the afoxolaner treated groups were administered the chewable formulation orally. Four sizes of chews were used containing 11.3 mg, 28.3 mg, 68 mg or 136 mg of afoxolaner. The dosing was administered as close as possible to the minimum effective dose of 2.5 mg/kg (ranging 2.5–3.11 mg/kg). Dogs were infested with 50 adult ticks of approximately equal sex ratio on the day prior to treatment (Day −1 or −2) and on Days 7, 14, 21, 28 and 35. Forty-eight hours after treatment and 48 h after each of the following weekly reinfestations, live ticks were removed and counted. Tick counts were performed by utilizing fingertips to locate the ticks, followed by visual categorization as alive/dead. After tick removal, a flea comb was applied to the area to ensure removal of all ticks (Marchiondo et al., 2013).

3. Data analysis

Total counts of live ticks were transformed to the natural logarithm (count +1) for calculation of geometric means by treatment group at each time point. Percent reduction from the control group mean was calculated for the treated group at each post-treatment time point 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. The log counts of the treated group were compared to the log counts of the untreated control group using an *F*-test adjusted for the allocation blocks used to randomize the animals to the treatment groups. The mixed procedure in SAS® version 9.1.3 was used for the analysis, with treatment group listed as a fixed effect and the allocation blocks listed as a random effect. The comparisons were performed using a two-sided test with a 5% significance level.

Table 2
Geometric mean tick counts on control dogs 48 h after treatment/infestation (minimum–maximum count per dog).

Days of infestation	Geometric mean tick count	
	Study A	Study B
−1 or −2	27.2 (20–39)	41.4 (29–54)
7	27.8 (16–37)	36.3 (26–47)
14	27.6 (6–42)[a]	33.9 (23–52
21	26.5 (13–44)	44.6 (34–61)
28	26.2 (10–45)[a]	44.1 (34–54)
35	28.5 (16–44)	43.6 (33–51)

[a] 7/8 dogs were infested with >12 ticks.

4. Results

Treated dogs in the two studies accepted the oral chewable of afoxolaner with no adverse reactions based on hourly post-treatment observations and daily observations. The control dogs were adequately infested by ticks in both studies (Table 2). According to Marchiondo et al. (2013) a minimum retention rate of ticks should be at least 20% in order to get a valid assessment of tick efficacy between a control and a treated group. The geometric mean tick infestations for the control dogs in the two studies ranged from 26.2 to 44.6.

In the two studies, afoxolaner was proven efficacious for treatment of existing or new tick infestations (Table 3).

Table 3
Efficacy of afoxolaner 48 h after treatment/infestation based on geometric mean tick counts.[a]

Day of tick infestation	% Reduction in live tick counts	
	Study A	Study B
−1 or −2	100	98.8
7	98.7	98.1
14	99.3	99.3
21	98.6	99.4
28	99.3	95.7
35	98.3	96.4

[a] There was a significant difference ($p < 0.001$) in tick counts between treated and control dogs at all time points up to Day 35.

Indeed, the curative efficacies were 98.8–100% against *R. sanguineus* within 2 days after treatment, while a prophylactic efficacy > 95.7% was maintained over five weeks. At all time points the difference in live tick counts between treated dogs and controls was statistically significant ($P < 0.001$).

5. Discussion

Within 48 h of treatment, afoxolaner oral formulation was highly efficacious against existing infestations by *R. sanguineus*. The two studies being independent, it was not possible to statistically compare the efficacies, but it does not seem to have any difference between the results observed on the South African and the Australian strains of *R. sanguineus*. The assessment of curative efficacy by counting existing ticks 48 h after treatment is a standard requirement (Marchiondo et al., 2013). This curative effect is demonstrated for the first time for an oral product whereas it is well known for many registered topical spot on formulations (Hunter et al., 2011; Kunkle et al., 2012). With regard to the curative efficacy, ticks are already attached and have started their blood meal when they are killed by acaricidal products. The situation differs between topical and oral products in the case of new tick infestations. In that case, topical ectoparasiticides acting by contact may kill ticks before attachment while attachment is a prerequisite for a systemic acting drug. In this study, reinfestations were also controlled for up to five weeks in the two studies, with efficacies greater than 95.7% at 48 h counts. This level of efficacy is similar to what has been published for topical formulations (Beugnet and Franc, 2012; Hunter et al., 2011; Kunkle et al., 2012). Afoxolaner is absorbed rapidly by the intestinal mucosa, and its plasma concentration peaks within 2–4 h after administration (Letendre et al., 2014), which ultimately results in a rapid uptake by the ticks. Further studies should be undertaken to assess the speed of kill on ticks after their attachment, knowing that ticks crawl on the skin of their host for a few hours before attaching. The oral route of administration offers several advantages over topical treatments. The efficacy of a formulation acting systemically is not affected by bathing, swimming, rain, or any skin condition. The dog owners can also handle their animal immediately.

These studies demonstrated that afoxolaner can be used as an effective agent to treat and control *Rhipicephalus* tick infestations with a convenient, monthly oral dosing schedule.

Conflict of interest

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

Blagburn, B.L., Dryden, M.W., 2009. Biology, treatment, and control of flea and tick infestations. Vet. Clin. North Am. Small Anim. Pract. 39, 1173–1200.

Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasites Vectors 3, 26.

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_guideline/2009/10/WC500004343.pdf

Gray, J., Dantas-Torres, F., Estrada-Pena, A., Levin, M., 2013. Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks Tick Borne Dis. 4, 171–180.

Hunter III, J.S., Baggott, D., Everett, W.R., Fourie, J.J., Cramer, L.G., Yoon, S.Y., Collidor, N., Mallouk, Y., Lee, L., Blair, J., Prullage, J.B., 2011. Efficacy of a novel topical combination of fipronil, amitraz and (S)-methoprene for treatment and control of induced infestations of brown dog ticks (*Rhipicephalus sanguineus*) on dogs. Vet. Parasitol. 179, 318–323.

Kunkle, B.N., Everett, W.R., Yoon, S.S., Beugnet, F., Pollmeier, M., 2012. Comparative curative efficacy of two spot on formulations, fipronil, amitraz, (S)-methoprene and imidacloprid–permethrin, on two tick species in dogs. Int. J. Appl. Res. Vet. Med. 10, 8–13.

Letendre, L., Huang, R., Kvaternick, V., Harriman, J., Drag, M., 2014. The intravenous and oral pharmacokinetics of afoxolaner used as a monthly chewable antiparasitic for dogs. Vet. Parasitol. 201, 190–197.

Marchiondo, A.A., Holdsworth, P.A., Fourie, L.J., Rugg, D., Kellmann, K., 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 I. Trends Parasitol. 25, 157–163.

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

Otranto, D., Dantas-Torres, F., Tarallo, V.D., Ramos, R.A., Stanneck, D., Baneth, G., de Caprariis, D., 2012. Apparent tick paralysis by *Rhipicephalus sanguineus* (Acari: Ixodidae) in dogs. Vet. Parasitol. 188 (3/4), 325–329.

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