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Assessment of the onset of action of afoxolaner against existing adult flea (*Ctenocephalides felis*) infestations on dogs

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ABSTRACT

The speed of kill of afoxolaner against experimental infestations by *Ctenocephalides felis* was evaluated after oral administration of afoxolaner in a soft chew (NEXGARD®) at a dose to achieve 2.5 mg/kg bodyweight. Forty beagles were allocated to two treatment groups. Dogs in Treatment Group 1 were untreated controls. Dogs in Treatment Group 2 were treated on Day-0 with afoxolaner, according to their pre-treatment bodyweight. All dogs were infested with approximately 100 *C. felis* on Day-1. Live fleas were counted upon removal at 5 time points after treatment (*i.e.*, 2, 4, 8, 12 and 24 h after treatment). For each time point, counts were performed on 4 dogs from each of the treated and the untreated groups. Early curative flea killing efficacy was evaluated with respect to the untreated control group. The afoxolaner treated group had significantly fewer fleas than the untreated control group at 8, 12, and 24 h ($p < 0.001$). The percent efficacies of orally administered afoxolaner were 15.0%, 87.8%, 99.5%, 100.0%, and 100.0% at 2, 4, 8, 12, and 24 h, respectively. In this study, afoxolaner began killing fleas by 2 h after treatment with increasing efficacy at subsequent time points and had >99.5% efficacy at 8, 12, and 24 h after treatment demonstrating an early onset of action.

1. Introduction

The burden of fleas is recognized for decades in companion animals worldwide. The cat flea, *Ctenocephalides felis felis*, is the predominant species found on dogs and cats (Beugnet and Franc, 2012; Dryden and Rust, 1994). Given the pathogenic and zoonotic potential and the high prevalence of fleas (Beugnet and Marie, 2009; Azad et al., 1997; Beugnet, 2013; Just et al., 2008), their control represents a key achievement for the health of cats and dogs. The optimal flea control program includes the rapid elimination of established flea infestations while providing a long lasting protection from a continuous challenge and at the same time demonstrating a high degree of efficacy. It also requires the quick elimination of adult fleas prior to egg production (Carlotti and Jacobs, 2000).

Afoxolaner is a recently identified insecticide-acaricide molecule belonging to the isoxazoline class that has demonstrated excellent effectiveness against fleas and ticks in dogs (Hunter et al., 2014; Dumont, 2014; Mitchell et al., 2014; Kunkle et al., 2014). It is formulated as an oral soft chewable and it acts systematically in the dog against fleas (Letendre et al., 2014). Afoxolaner is a specific and novel blocker of ligand-gated chloride channels in insects, resulting in hyperexcitation and rapid death of the arthropods (Shoop et al., 2014).

We herein provide additional data on the efficacy of afoxolaner against *C. felis*. The study was conducted to determine the curative speed of kill against existing adult flea infestations on dogs.

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Table 1
Percent curative efficacy of oral formulation of afoxolaner administered to dogs against existing *C. felis* flea infestations at minimum therapeutic dose of 2.5 mg/kg.

Time after treatment (h)	*N*	Untreated control geometric mean flea count	*N*	Treated oral geometric mean flea count	Percent efficacy	*p*-Value
2.0	4	79.2	4	67.4	15.0	0.209
4.0	4	74.3	4	9.0	87.8	0.064
8.0	4	65.3	4	0.3	99.5	<.001
12.0	4	67.2	4	0.0	100.0	<0.001
24.0	4	64.5	4	0.0	100.0	<0.001

2. Materials and methods

2.1. Animals

Forty-four healthy beagles of both sexes (22 males and 22 females), 13.8–37.5 months of age, and weighed 7.05–14.75 kg were included in the study. The protocol of the study was reviewed and approved by the Merial Institutional Animal Care and Use Committee (IACUC). Dogs were handled with due regard for their welfare (USDA, 2008). All animals were housed individually. All dogs received commercial food, once daily, in a sufficient amount to maintain body weight appropriate for the breed, and water was provided *ad libitum*. The dogs were not treated with ectoparasiticides (either topical or systemic) within three months prior to the start of the study. Dogs enrolled in the studies underwent a full physical examination by a veterinarian on Day-7 and were examined once daily for health observations.

3. Experimental study designs

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 Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guideline GL9 (EMEA, 2000).

The study was a blinded, negative controlled study using a randomized block design with blocks of 4 dogs based on the flea counts obtained after preliminary infestations on Day-6 for allocation purposes. The 4 dogs with the lowest pre-infestation flea counts were not allocated. The *C. felis* strain used for infestations was a U.S. strain of fleas that has been maintained for approximately 6 years from fleas collected in Oklahoma (Ecto Services, Inc.).

Each infestation (Day-6 for allocation purpose and Day-1 for treatment evaluation), was performed by placing approximately 100 (±5) *C. felis* (equal numbers of male and female adult fleas) along the dorsum or the dorso-sacral area of each dog.

Within each block, the dogs were randomized to one of the 10 counting time-by-treatment combinations as follows: 2 h: untreated control and treated; 4 h: untreated control and treated; 8 h: untreated control and treated; 12 h: untreated control and treated; 24 h: untreated control and treated.

Dogs in the treated group were dosed orally on Day-0 with the appropriate chewable tablets containing afoxolaner. 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. The dose range was 2.5–2.97 mg/kg using a combination of the chews in order to be as close as possible to the minimum therapeutic dose of 2.5 mg/kg. Dogs were observed prior to treatment and hourly (±30 min) for 4 h post-treatment. At 2, 4, 8, 12 and 24 h after oral treatment depending on the groups, animals were combed and fleas were removed, counted and categorized as dead or alive.

4. Data analysis

The flea counts were transformed to the natural logarithm of (count + 1) for calculation of geometric means by treatment group at each time point. Percent efficacy of the treated group with respect to the control group was calculated using the formula $[(C-T)/C] \times 100$, where C = geometric mean for the control group and T = geometric mean for the treated group for each time point. 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 at each time point separately. 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. All comparisons were made using the (two-sided) 5% significance level.

5. Results

No adverse events related to the administration of afoxolaner soft chewables were observed during the study. The onset of efficacy of orally administrated afoxolaner on pre-existing flea infestations are presented in Table 1. The percent efficacies for the treated groups were 15%, 87.8%, 99.5% 100%, and 100% at 2, 4, 8, 12 and 24 h, respectively. The treated dogs had fewer fleas than the untreated control group at 4 h and significantly fewer at all following time points ($p \leq 0.001$). In this study, the oral administration of afoxolaner provided a significant reduction in the flea burden by 4 h after treatment and

reached a high flea killing activity by 8 h after administration.

6. Discussion

This study demonstrated that afoxolaner administered in a beef-flavored soft chew at the minimum therapeutic dose of 2.5 mg/kg, provided a rapid adulticidal efficacy (87.8% within 4 h compared to the control dogs). The efficacy of afoxolaner compared to control dogs increased to 99.5% at 8 h post-treatment and 100% at 12 and 24 h post-treatment.

The efficacy results correlate with the pharmacokinetic data that indicates that following administration of 2.5 mg/kg to dogs, afoxolaner plasma concentrations increased rapidly to peak within 2–6 h. The flea and tick efficacies are directly related to the rapid absorption of afoxolaner and a plasma peak reaching the lethal concentration (LC90) in the blood, around 23 ng/mL for fleas and 100 ng/mL for ticks (Letendre et al., 2014; Shoop et al., 2014). The speed of kill is also be related to the speed and number of fleas taking a blood meal soon after the oral dosing of the dogs. In addition, the long terminal plasma half-life of approximately two weeks (12.8 ± 5.6 days) results in an average afoxolaner plasma concentration above the effective concentration for efficacy against fleas for a duration of one month (Letendre et al., 2014). This long lasting efficacy has been confirmed in several experimental studies conducted on *C. canis* and *C. felis* fleas (Dumont, 2014; Hunter et al., 2014).

Conflict of interest statement

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

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References

Azad, A.F., Radulovic, S., Higgins, J.A., Noden, B.H., Troyer, J.M., 1997. Flea-borne rickettsioses: ecologic considerations. Emerg. Infect. Dis. 3, 319–327.

Beugnet, F., Marie, J.L., 2009. Emerging arthropod borne diseases of companion animals in Europe. Vet. Parasitol. 163, 298–305.

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., 2013. Guide to Vector Borne Diseases of Pets. Ed Merial, France, pp. 425.

Carlotti, D.N., Jacobs, D.E., 2000. Therapy, control and prevention of flea allergy dermatitis in dogs and cats. Vet. Dermatol. 11, 83–98.

Dryden, M.W., Rust, M.K., 1994. The cat flea: biology, ecology and control. Vet. Parasitol. 52, 1–19.

Dumont, P., 2014. Curative and preventative efficacy of orally administered afoxolaner against dog flea *Ctenocephalides canis* (Curtis, 1826) infestation in dogs. Vet. Parasitol. 201, 212–215.

EMEA, 2000. Guideline on Good Clinical Practices. The European Agency for the Evaluation of Medicinal Products. VICH Topic GL9 (GCP). (EMWA/CVMP/VICH/595/98-Final).

Hunter III, J.S., Dumont, P., Chester, T.S., Young, D.R., Fourie, J.J., Larsen, D.L., 2014. Evaluation of the curative and preventive efficacy of a single oral administration of afoxolaner against cat flea *Ctenocephalides* felis infestations on dogs. Vet. Parasitol. 201, 207–211.

Just, F.T., Gilles, J., Pradel, I., Pfalzer, S., Lengauer, H., Hellmann, K., Pfister, K., 2008. Molecular evidence of *Bartonella* spp. in cat and dog fleas from Germany and France. Zoo. Public Health 55 (8–10), 514–520.

Kunkle, B., Daly, S., Dumont, P., Drag, M., Larsen, D., 2014. Assessment of the efficacy of orally administered afoxolaner against Rhipicephalus sanguineus sensu lato. Vet. Parasit. 201, 226–228.

Letendre, L., Huang, R., Kvaternick, J., Drag, M., 2014. The intravenious and oral pharmacokinetics of afoxolaner used as a monthly chewable antiparasitic for dogs. Vet. Parasitlol. 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.

Mitchell, E.B., McCall, J.W., Chester, S.T., Larsen, D., 2014. Efficacy of afoxolaner against Ixodes scapularis ticks in dogs. Vet. Parasitol. 201, 223–225.

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.

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