Evaluation of the curative and preventive efficacy of a single oral administration of afoxolaner against cat flea *Ctenocephalides felis* infestations on dogs

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**ABSTRACT**

The efficacy of orally administered afoxolaner for treatment and prevention of repeated infestations with adult *Ctenocephalides felis* on dogs was evaluated in two studies after administration of a beef-flavored soft chew. In each study, 32 dogs were divided randomly into four equal groups. Dogs in Groups 1 and 3 were not treated and served as controls. Dogs in Groups 2 and 4 were treated on Day 0 with a combination of chewable tablets to be as close as possible to the minimum therapeutic dose of 2.5 mg/kg. All animals were infested experimentally with unfed *C. felis* (100 ± 5) on Days −1, 7, 14, 21, 28 and 35. Flea killing efficacy was evaluated in both studies while, efficacy against flea egg production was assessed in Study 1. Live fleas were counted at 12 (Groups 1 and 2) and 24 h (Groups 3 and 4), after treatment or after weekly infestations. In Study 1, flea eggs were collected and counted at either 12 or 24 h after each flea infestation on Days 7, 14, 21, 28 and 35. The results of both studies demonstrate the long lasting and rapid efficacy of afoxolaner against *C. felis*, when administered as a single oral dose to dogs. For flea counts conducted 24 h after treatment or infestation, efficacy was 100% for all time points up to Day 36 in both studies, except for one time point (99.9% on Day 22) for Study 2. For flea counts performed 12 h after treatment or infestation, efficacy was >95.2% until Day 21 in both studies. Efficacy at 12 h was >93.0% on Day 35 in Study 1 and >89.7% on Day 35 in Study 2. The treated groups had significantly fewer fleas than untreated control dogs in both studies for all flea counts (*p* = 0.003 Study 1, *p* = 0.0006 Study 2). In Study 1, for all egg counts performed at or beyond Day 7, efficacy in egg reduction was >99% for all time points between Days 7 and 35.

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1. **Introduction**

Ectoparasitoses account for the most frequent diseases in carnivores worldwide with fleas and ticks representing the most prevalent parasites (Beugnet and Franc, 2012; Otranto et al., 2009a, b). The cat flea, *Ctenocephalides felis*, is the main flea species infesting both dogs and cats (Dryden and Rust, 1994; Rust and Dryden, 1997). In addition to causing annoyance and discomfort to pets and their owners, cat fleas are associated with several diseases. *C. felis* is primarily responsible for flea bite allergy dermatitis (FAD) in dogs and...
cats (Dryden and Blakemore, 1989; Plant, 1991; Carlotti and Costargert, 1994) as a result of hypersensitivity to components of flea saliva (Dryden and Rust, 1994; Stopler, 1994). The cat flea is also the primary intermediate host of the tapeworm Dipylidium caninum, the common intestinal cestode of dogs and cats (Dunn, 1978; Pugh, 1987). *C. felis* can also transmit murine typhus, *Rickettsia felis*, and has been implicated in the transmission of some *Bartonella* species, such as *B. henselae*, the agent of Cat Scratch Disease (Azad et al., 1997; Orloski and Lathrop, 2003; Just et al., 2008).

Although the use of insecticides such as fipronil, imidacloprid, selamectin, and spinosad has revolutionized flea control in recent years, treatment and prevention of cat flea infestations remain a major concern for pet owners and veterinarians (Beugnet and Franc, 2012; Rust, 2005). New compounds that are fast acting, long lasting, and easy to administer are needed to complement the existing products on the market. This paper demonstrates the efficacy of orally administered afoxolane, a newly identified isoxazoline insecticide–acaricide molecule (Letendre et al., 2014; Shoop et al., 2014) against *C. felis* in dogs.

2. Materials and methods

2.1. Animals

Both studies were conducted with thirty-two healthy beagles of both sexes. The dogs in Study 1 included twenty-two males and ten females over twelve months of age which weighed between 9.1 and 19.1 kg. The dogs allocated to Study 2 were twelve males and twenty females over 6 months of age which weighed between 8.2 kg and 19.6 kg. The protocol of the studies was reviewed and approved by the Merial Institutional Animal Care and Use Committee. 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 a healthy physical state, 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.

2.2. Experimental study designs

The study designs were 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, (VICH guideline GL9) (EMEA, 2000).

The studies were blinded, negative-controlled clinical efficacy studies, utilizing a block design based on Day −6 flea counts. In each study, dogs were infested with 100 (±5) unfed adult *C. felis* on Day −7, which were removed and counted on Day −6. The *C. felis* strain used for infestations in Study 1 was a U.S. strain of fleas that has been maintained for approximately 11 years from wild fleas captured in California. In Study 2, a flea strain that originated from Hanover University, Germany, was used. The experimental study unit was the individual dog, which was identified, treated and assessed on an individual basis. In each study, the 32 dogs were allocated to 8 blocks of 4 animals each. Within each block, the dogs were randomly assigned to one of the 2 counting time-by-treatment combinations (*i.e.*, control 12-h (Groups 1), treated 12-h (Groups 2), control 24-h (Groups 3) and treated 24-h (Groups 4).

In each study, dogs were infested with 100 (±5) unfed adult *C. felis* once on Days −1, 7, 14, 21, 28 and 35. Fleas were removed, counted and categorized as dead/alive 12 h ± 30 min after treatment or challenge infestation for Groups 1 and 2 and 24 ± 1 h after treatment or challenge infestation for treatment Groups 3 and 4. Each dog was combed for a minimum of 10 min using a fine-toothed flea comb. If fleas were found during these 10 minutes, then the dog was combed for 15 min. However, if no fleas were found on the dog in these 10 min, then the combing was stopped (Marchiondo et al., 2013). The personnel performing comb counts and caring for the animals were blinded to all treatment group allocations.

In addition in Study 1, collections of flea eggs were also performed on Days 7, 14, 28 and 35 (at +12 h and +24 h). TheASTE below the cages were cleaned, and the pans were lined with paper that facilitated the collection of flea eggs. The shed flea eggs were collected and counted at 12 h ± 30 min post infestation for Groups 1 and 2 and at 24 ± 1 hour post infestation for Groups 3 and 4. Debris was sifted in a 30-mesh strainer and the eggs were retained. Collected eggs were counted and recorded.

In Study 1, any remaining food was removed from the dogs in the afternoon of Day −1, and dogs were not fed prior to treatment on Day 0, whereas in Study 2, dogs were either offered their normal ration prior to treatment or immediately following treatment on Day 0. In both studies, dogs in Groups 1 and 3 remained untreated and served as controls. In both studies, dogs in Groups 2 and 4 were dosed once orally with the appropriate soft chew formulations containing afoxolane. 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 afoxolane. The dose range was 2.5–3.1 mg/kg in Study 1 (mean = 2.76 mg/kg in Group 2 and 2.83 mg/kg in Group 4) and 2.5–2.8 mg/kg in Study 2 (mean = 2.63 mg/kg in Group 2 and 2.66 mg/kg in Group 4) 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.

2.3. 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 

\[ \frac{|C - T|}{C} \times 100 \]

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.

The egg counts at each time point 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.

The log-counts of the two groups were compared using the mixed procedure in SAS® version 9.1.3 with treatment as the fixed effect and allocation block the random effect. A two-sided 5% significance level was used.

3. Results

3.1. Flea counts

Table 1. Efficacy of afoxolaner soft chew (2.5 mg/kg) against adult fleas (Ctenocephalides felis) evaluated 12 or 24 h after treatment of dogs and after subsequent challenges for 5 weeks.

<table>
<thead>
<tr>
<th>Study 1</th>
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<tbody>
<tr>
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</tr>
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3.2. Eggs counts

The flea egg counts for Study 1 are summarized in Table 2.

Table 2. Efficacy of afoxolaner soft chew (2.5 mg/kg) against adult fleas (Ctenocephalides felis) evaluated 12 or 24 h after treatment of dogs and after subsequent challenges for 5 weeks.

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3.1. Flea counts

The flea counts for both studies are summarized in Table 1. In both studies, the flea counts of untreated dogs were consistently high, with geometric means ranging from 58.9 to 93.8.

For live flea counts performed 12 h after treatment or challenge infestations, the percent efficacy was \(\geq 95.2\)% for the first four infestations through Day 21, was 81.1% on Day 28, and was 93% on Day 35 in Study 1. Efficacy at 12 h was \(\geq 98.5\)% through Day 28 and 89.7% on Day 35 for Study 2.

For live flea counts performed 24 h after treatment or challenge infestations, percent efficacy was 100% for all time points until Day 35, with the exception of one time point (99.9% on Day 22) for Study 2. The flea counts were significantly different between treated and control dogs at all time-points for both 12 and 24 h counts in both studies \((p = 0.003\) Study 1, \(p = 0.0006\) Study 2).

3.2. Eggs counts

The flea egg counts for Study 1 are summarized in Table 2.

For egg counts performed 12 h after treatment, meaning 36 h after flea infestations, 22 eggs were found from treated dogs (Groups 2, 0–11 eggs per dog) and a total of 183 eggs from control dogs (Groups 1, 4–90 eggs per dog) (efficacy of 88.8%, \(p < 0.004\)). For egg count performed 24 h after treatment, meaning 48 h after flea infestations, 43 eggs were found from treated dogs (Groups 4, 1–17 eggs per dog) and a total of 431 eggs from control dogs (0–118 eggs per dog) (efficacy of 85.8%, \(p = 0.028\)).

For egg counts performed 12 h after infestation at or beyond Day 7, two treated animals had a single egg collected on Day 14, compared to 216 eggs collected in Group 1 dogs (9–62 eggs per dog), resulting in 99.1% efficacy. One treated animal had a single egg collected on Day 21, compared to 213 eggs collected in Group 1 dogs (3–81 eggs per
dog) resulting in 99.5% efficacy. No eggs were collected on treated dogs at Days 7, 28 or 35, when 150, 194 and 112 eggs were collected from control dogs at Days 7, 28 and 35, respectively.

For *C. felis* egg counts performed 24h after challenge infestation at or beyond Day 7, only one treated animal had a single egg collected on Day 15, compared to 438 eggs collected from control dogs (8–113 eggs per dog), resulting in 99.8% efficacy. For all other time points at 24h post-infestation, the efficacy was 100% as no eggs were found in the treated Group. In the control group, 297, 421, 253 and 357 eggs were collected at Days 8, 22, 29 and 36, respectively.

There was no observed adverse event based on hourly post-treatment observations for 4 h and daily observations thereafter.

### 4. Discussion

Oral treatment of dogs with the new insecticide afoxolaner at the minimum effective dose of 2.5 mg/kg provided ≥99.9% efficacy against adult fleas at 24h count for 5 weeks after a single administration.

The present study also demonstrated a sustained 12 h efficacy against adult fleas for a full month. The results of additional efficacy studies (Kunkle et al., 2014) demonstrated the excellent activity of afoxolaner against the dog flea *Ctenocephalides canis* (Dumont, 2014).

In addition, the results of flea egg counts demonstrated that one treatment reduced egg production by 99.1–100% as early as 12 and 24h after infestation for up to 5 weeks.

The ability to block egg production is an especially important consideration since adult female *C. felis* can lay an average of 20–30 eggs a day (Guaguère and Beugnet, 2008). Thus, the regular use of afoxolaner may potentially reduce household contamination by flea eggs and, consequently, the flea biomass in the environment.

Interestingly, according to the design of the present study, the presence of flea eggs in the control group was uncertain since it is usually accepted that female fleas start laying eggs on average 36 h after the host infestation (Dryden and Rust, 1994). Nevertheless, a sufficient eggs production was obtained in the control group as early as 12h (112–213 eggs) and 24h (253–421 eggs) after each infestation. This early egg laying may be related to potential reproductive specificity of the *C. felis* strain used for infestations. Nevertheless, a similar early egg production was obtained with a *C. canis* flea strain in another study (Dumont, 2014). It rather suggests that some females in any flea populations may start to lay eggs sooner after host infestation than previously assumed. This later characteristic underlines the need for insecticides with a sufficient speed of action allowing the killing of fleas before they start laying eggs. Alternatively the combination of an IGR (Insect Growth Regulator) with the insecticide will prevent the development of immature flea stages (Beugnet et al., 2012).

In conclusion, these studies confirm the excellent efficacy of a single treatment of afoxolaner in beef-flavored soft chews against *C. felis* and also provide evidence that monthly treatments with afoxolaner can prevent *C. felis* infestations by killing the adults before egg production starts.

### 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


