Veterinary Parasitology 201 (2014) 220–222

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Efficacy of afoxolaner against *Dermacentor variabilis* ticks in dogs

Elizabeth B. Mitchell[a,*], Paul Dorr[a], William R. Everett[b], Theodore S. Chester[a], Diane Larsen[a]

[a] Merial Limited, 3239 Satellite Boulevard, Duluth, GA 30096, USA
[b] BerTek, Inc., 104 Wilson Bottoms Road, Greenbrier, AR 72058, USA

ARTICLE INFO

Keywords:
Afoxolaner
Dogs
Dermacentor variabilis
Ticks

ABSTRACT

Efficacy of afoxolaner, a novel isoxazoline insecticide/acaricide, against *Dermacentor variabilis* ticks was confirmed in two laboratory studies. Each study utilized a controlled, randomized block design. One day prior to treatment, beagle dogs were infested with 50 unfed adult ticks. Repeat infestations were performed weekly for four weeks. The number of live ticks remaining on each dog was determined 48 h after treatment and after each subsequent infestation. A single oral treatment with a dose approaching the minimum effective dose of afoxolaner (2.5 mg/kg) eliminated the pre-existing infestations by *D. variabilis* ticks and controlled weekly re-infestations with 99.7–100% efficacy up to Day 23 and >97% efficacy at Day 30.

1. Introduction

Tick control is an important concern for public health officials, pet owners, and veterinarians (Dantas-Torres et al., 2012; Otranto and Wall, 2008). Indeed, tick infestations can be a nuisance, and heavy tick infestation can lead to anemia, particularly in young or small dogs. *Dermacentor variabilis*, generally known as 'American dog tick', is one of the most common tick species affecting dogs in the USA (Dryden and Payne, 2004; Goldberg et al., 2002). *D. variabilis* is widely distributed across the central and eastern United States, and also occurs along the Pacific coast, and it is an important vector of several infectious agents, including those that cause Rocky Mountain spotted fever, tularemia, and ehrlichiosis in both dogs and humans (Chomel, 2011; Dantas-Torres et al., 2012; Dryden and Payne, 2004; Steiert and Gilfoy, 2002). *D. variabilis* is also commonly implicated as a cause of tick paralysis (Vedanarayanan et al., 2004). Currently, tick control for dogs is only available in formulations that are topically applied (Sprays, powders, shampoos, spot ons) or in collars (Beugnet and Franc, 2012). Afoxolaner is a novel insecticide–acaracide that is administered orally in a chewable formulation (Nexgard®, Merial). Afoxolaner is a member of the isoxazoline class and works by inhibiting insect GABA and Glutamate-gated chloride channels (Shoop et al., 2014), thereby leading to prolonged hyper-excitation and death of both insects and acarines. This paper describes two studies that were performed to demonstrate the efficacy of afoxolaner against *D. variabilis* ticks.

2. Materials and methods

2.1. Experimental design

Two similar studies were conducted to demonstrate the efficacy of afoxolaner against *D. variabilis*. Both studies were performed in the USA and were designed in accordance with standard methods for evaluating the

* Corresponding author. Tel.: +1 706 552 2782; fax: +1 706 552 2451.
E-mail address: Elizabeth.Mitchell@Merial.com (E.B. Mitchell).

http://dx.doi.org/10.1016/j.vetpar.2014.02.016

Table 1
Study design to evaluate the efficacy of afoxolaner against *Dermacentor variabilis*.

Study	Subject sex	Subject age (months)	Subject body weight (kg)	Target	Treatment[a]	Challenge infestations: tick no.[b]	Challenge infestations: study days	Tick count days
1	6 males, 10 females	6–8	5.7–9.1	*Dermacentor variabilis*	Once on Day 0	50 ± 5	−1, 7, 14, 21, 28, 35	2, 9, 16, 23, 30, 37
2	10 males, 6 females	7–10	5.9–9.6	*Dermacentor variabilis*	Once on Day 0	50 ± 5	−1, 7, 14, 21, 28, 35	2, 9, 16, 23, 30, 37

[a] Treated dogs were given the test medication orally at a minimum dosage of 2.5 mg/kg (range 2.57–3.96 mg/kg).
[b] Tick no. = number of ticks used for infestation of each dog on each day.

efficacy of parasiticides for the treatment, prevention and control of tick infestations (Marchiondo et al., 2013). The studies were approved by the Merial Institutional Animal Care and Use Committee. Dogs were managed consitent with the US Animal Welfare Regulations (USDA, 2008).

2.2. Animals

The studies involved 32 purpose bred beagles (16 per study) which were individually identified by unique ear tattoos. Study 1 included 6 male and 10 female dogs aged 6–8 months and weighing 5.7–9.1 kg. Study 2 included 10 male and 6 female dogs aged 7–10 months and weighing 5.9–9.6 kg (Table 1). Studies followed a controlled, randomized design. Dogs were in good health and had not been treated with ectoparasiticides for at least 3 months prior to treatment. Tick infestations and subsequent counts were performed prior to treatment, and confirmed that dogs were capable of maintaining adequate infestations. Dogs were housed individually. Health observations were conducted daily throughout each study. In addition, hourly health observations were conducted for 4 h following treatment with afoxolaner on Day 0.

2.3. Study design

For both studies, seven days prior to treatment (designated as to Day 0) dogs were infested with 50 adult ticks of approximately equivalent sex ratio, which were removed and counted 48 h later. The dogs were ranked in order of these pre-treatment tick counts (highest to lowest). The first two dogs were assigned to Block 1, the next two dogs to Block 2 and so on until 10 blocks of two dogs each were formed. Within blocks, dogs were randomly assigned to one of two treatment groups. Dogs in Group 1 were untreated controls. Dogs in Group 2 were treated once orally with the appropriate combination of soft chewables containing afoxolaner. Two sizes of chews were used: 0.5 g and 1.25 g, containing 11.3 mg and 28.3 mg of afoxolaner, respectively. The soft chewables are not designed to be divided, therefore, the dosing was administered as closely as possible to the minimum effective dose of 2.5 mg/kg using whole chews. The doses administered to dogs ranged from 2.57 to 3.96 mg/kg body weight in Study 1 and from 2.97 to 3.70 mg/kg body weight in Study 2. The dogs were observed during the 4 h following their treatment and daily throughout the study.

Dogs were infested with 50 adult ticks (25 females and 25 males) on the day prior to treatment (Day – 1) and on Days 7, 14, 21, and 28. Forty-eight hours after treatment and 48 h after each of the subsequent re-infestations, ticks were removed and live ticks were counted. These counts were conducted using a procedure involving methodical examination of all body areas using finger tips and/or a coarse tooth comb to sort through the hair and locate all ticks following WAAVP guidelines (Marchiondo et al., 2013). The two studies used unfed adult *D. variabilis* ticks from two separate laboratory-maintained populations. Each laboratory population had been established from ticks collected in the USA. Personnel responsible for collection of animal health and efficacy data were blinded to the treatment groups.

2.4. Data analysis

Total counts of live ticks were transformed to the natural logarithm of (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 comparisons were performed using a two-sided test with a 5% significance level.

3. Results

Treated dogs in both studies accepted the afoxolaner chews based on hourly post-treatment observations for four hours and daily observations thereafter, and no vomiting was reported in treated dogs during either of the studies. No treatment-related health problems were observed throughout the studies. At all time-points in both studies the retention rates of ticks in the untreated control dogs exceeded the 20% attachment rate recommended by Marchiondo et al. (2013) to allow a robust comparison between control and treated groups (Table 2).

In dogs infested one day prior to treatment, afoxolaner provided 100% curative efficacy against *D. variabilis* within 48 h following treatment in both studies (Table 2). Dogs were re-infested on a weekly basis up to Day 28, and in the two studies the efficacy 48 h after re-infestation remained at 100% up to Day 16 and >97.3% up to Day 30 (Table 2). There was a significant difference ($p < 0.001$) between treated and control dogs for counts of ticks at all time-points up to Day 30.

Table 2
Geometric mean and range of live *Dermacentor variabilis* tick counts on control dogs 48 h after treatment (Day – 1 infestation) or after subsequent re-infestations, and percent efficacy based on geometric means.

Day of infestation	Day of tick count	Study 1 Geometric mean counts in control dogs (range)	Study 1 Percent efficacy based on geometric means[a]	Study 2 Geometric mean counts in control dogs (range)	Study 2 Percent efficacy based on geometric means[a]
−1	2	40.1 (32–52)	100	40.0 (27–50)	100
7	9	40.3 (33–50)	100	29.1 (15–50)	100
14	16	33.3 (27–47)	100	26.2 (14–47)	100
21	23	33.9 (24–43)	99.7	27.0 (11–45)	99.7
28	30	29.3 (17–47)	97.3	32.7 (21–42)	98.5

[a] There was a significant difference ($p < 0.001$) in tick counts between treated and control dogs at all time-points up to Day 30.

4. Discussion

Afoxolaner was highly effective in eliminating existing infestations of *D. variabilis*, demonstrating 100% effectiveness within 48 h following a single oral treatment. Afoxolaner also provided extended efficacy following re-infestation with *D. variabilis* ticks, with >97.3% efficacy for one month after treatment. The excellent efficacy of afoxolaner against *D. variabilis* for an entire month after treatment is predicted by the plasma concentration profile of afoxolaner, which remains above the EC_{90} for *D. variabilis* (afoxolaner plasma concentration at which there is 90% effectiveness) past 30 days after administration of the minimum dose of 2.5 mg/kg body weight (Letendre et al., 2014).

There is no direct comparison with other acaricidal treatment, but it is possible to look at the published results of tick efficacy studies using a comparable design. The acaricidal effect of afoxolaner in the two studies presented here was close to that of topical products reported in the literature. Rugg et al. (2007) reported two studies evaluating efficacy of topical products against *D. variabilis*. In one of these studies the efficacy of a topical product containing metaflumizone + amitraz against *D. variabilis* ticks 48 h after infestation was 89.4% on Day 28 (Rugg et al., 2007). The second study evaluated the efficacy of a topical product containing metaflumizone + amitraz as well as a topical product containing fipronil + S-methoprene against *D. variabilis* ticks 48 h after infestation and reported efficacy of 89.8 and 93.8% respectively at Day 28 (Rugg et al., 2007). This is the first time that an oral formulation provides a month long efficacy against tick that is comparable to acaricidal spot-on products.

Pet owners may prefer the option of treating their dog with an oral medication that provides both flea and tick control compared to a topical application. The efficacy of some topical products may be affected by bathing or swimming, or there may be a period of time in which the application site should be avoided by pet owners (Blagburn and Dryden, 2009).

Nexgard® (afoxolaner) is currently the only oral product that kills adult fleas and treats several tick species including the American dog tick.

Conflict of interest

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

Acknowledgments

All studies were funded by Merial Limited. The authors gratefully acknowledge the staff at BerTek, Inc. (Greenbrier AR, USA) and at Merial Limited for their help in conducting the studies to a high professional standard. The authors gratefully acknowledge Michael Murray and Frederic Beugnet for the scientific editing of the manuscript.

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