Evaluation of the efficacy of afoxolaner against *Haemaphysalis longicornis* on dogs

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**A R T I C L E   I N F O**

Keywords:

*Haemaphysalis longicornis*  
Tick  
Afoxolaner  
Dog  
Efficacy

**A B S T R A C T**

A controlled study to assess the acaricidal efficacy of afoxolaner in dogs after a single oral administration was conducted against *Haemaphysalis longicornis* ticks. The study was characterized by a negative controlled randomized block design and included sixteen beagle dogs of both sexes. Starting two days before treatment, each dog was infested weekly with 50 ticks over 4 weeks. The number of live ticks was determined 48 h after treatment and then 48 h after each infestation. The mean dose of afoxolaner received by dogs was 3.0 mg/kg (range: 2.5–3.1 mg/kg). Afoxolaner rapidly eliminated pre-existing tick infestations (100% ticks killed within 48 h of treatment) and controlled weekly re-infestations (91.9% prophylactic efficacy at Day 30).

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1. Introduction

*Haemaphysalis longicornis* is common tick species in Asia and Pacific region, including Japan, China, Korea, Australia and New Zealand (Inokuma, 2013; Shimada et al., 2003; Tenquis and Charleston, 2001). This species is the major vector of *Babesia gibsoni* to dogs in Asia (Chomel, 2011; Inokuma, 2013). Canine babesiosis is an important tick-borne disease, and the prevention of *Babesia* transmission is particularly critical given the challenges of appropriate treatment strategies against babesiosis (Beugnet and Franc, 2012; Otranto and Wall, 2008; Otranto et al., 2009a,b; Taboada and Lobetti, 2006). *H. longicornis* is also a putative vector of *Hepatozoon canis* and *Rickettsia japonica* (Inokuma, 2013).

The present study describes the result of a laboratory study that assessed the efficacy of afoxolaner, administered orally in a chewable formulation (Nexgard\(^\text{®},\) Merial), against *H. longicornis* in dogs.

2. Materials and methods

Sixteen beagle dogs of both sexes were included in the study, which was designed as a negative controlled randomized block study. All dogs were approximately 10–11 months of age and weighed from 7.2 to 9.0 kg at inclusion.

All dogs were healthy and had not been treated with any ectoparasiticides in the 3 months prior to inclusion in the study nor infested by ticks. The health of all dogs was monitored at least once daily and once per hour during the first four hours post treatment. All dogs had free access to water and were fed a commercial diet. The study design was approved by the Merial Institutional Animal Care and Use Committee (USDA, 2008).

In the study, two groups of eight dogs were formed randomly, using blocks of two dogs based on decreasing...
pre-treatment tick counts. Dogs in Group 1 were untreated controls. Dogs in Group 2 were treated 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. Doses were administered as closely as possible to the minimum effective dose (2.5 mg/kg). In this study, all dogs received 2 chewable tablets of 0.5 g, and the mean dose received by dogs was 3.0 mg/kg of afoxolaner (range: 2.5–3.1 mg/kg).

On Days 2, 7, 14, 21, and 28, all dogs were infested with 50 unfed female *H. longicornis*. The study utilized ticks from laboratory-maintained populations that had been established from ticks collected in field locations in Japan. Ticks were counted 48 h after treatment (Day 2) or after infestations on Days 9, 16, 23, and 30. Counting of ticks on the dogs was performed by parting and feeling through dog’s hair with finger tips. All personnel conducting tick counts and health observations were blinded to treatment groups. 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 compliance with VICH GL9 “Good Clinical Practice” (EMEA, 2000).

For each tick count, the total count of live ticks was transformed to the natural logarithm (count +1) to calculate the geometric mean for each treatment group. The percent reduction of the live 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 log-counts of the live ticks 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. All testing was two-sided at the significance level \(p = 0.05\).

3. Results

At each time point, the geometric mean counts of the live ticks in the control group ranged between 13.9 and 23.7 (Table 1). This level of infestation in the control group was adequate for determining efficacy against ticks. The WAAVP guideline recommends that at least 20% of the ticks should be retained from the infestation, meaning an average of 10 ticks per dog out of the 50 used to infest each dog (Marchiondo et al., 2013).

The curative efficacy of afoxolaner against pre-existing tick infestation was 100% at 48 h after treatment (Table 1). The tick efficacy of afoxolaner on weekly re-infestations starting on Day 7 after treatment provided 100% acaricidal efficacy at Day 9 and over 91.9% efficacy at all subsequent time points up to Day 30 (Table 1). The tick counts were significantly different in treated and control dogs at all time points \((p < 0.05)\).

One dog in the untreated control group was removed from the study on Day 21 due to mild seizures observed on Day 17 and Day 21. All data on the dog captured prior to removal were included in the analysis. No adverse reaction to the treatment was observed during this study.

4. Discussion

The attachment rate of the ticks was lower than what is usually observed for other tick species infesting dogs such as *Rhipicephalus sanguineus* (Kunkle et al., 2014). This is known to occur for *H. longicornis*, which is more adapted to wild ruminants than dogs in the adult stage (Inokuma, 2013; unpublished data). In this experiment, 4 ticks at Day 2 and 6 ticks at Day 16 were recovered from one control dog. It is below the 20% of attachment rate proposed by Marchiondo et al. (2013). Nevertheless, the geometric mean count values ranged from 13.9 to 23.7 which were above the 20% attachment rate. The geometric mean counts were used to calculate the percent efficacy, which is therefore valid based on the guidelines (Marchiondo et al., 2013).

A single oral treatment with the chewable formulation of afoxolaner achieved 100% curative efficacy for treating preexistant infestation of *H. longicornis*. It also controlled re-infestation by eliminating >90% of the ticks within the first 48 h after infestation for 4 weeks after treatment. The Day 30 efficacy of afoxolaner seems lower than what has been observed for other tick species (Kunkle et al., 2014; Mitchell et al., 2014). This may be related to a lower attachment rate in the control dogs, but also the variability of each experiment.

There are some reports on the efficacy of current veterinary topical products against *H. longicornis*, e.g. imidacloprid/permethrin, fipronil/(S)-methoprene (Hagimori et al., 2005). However, unlike these topical combinations, ticks are exposed to afoxolaner while feeding on the host’s blood. 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 rapid uptake of the active ingredient by the ticks and a tick efficacy assessed at 48 h is similar to that of imidacloprid/permethrin or fipronil/(S)-methoprene combinations (Hagimori et al., 2005).

The systemic distribution offers advantages compared to topical formulations. There is no period after treatment administration during which the application site should be avoided by the owners and wetting of the haircoat or any

<table>
<thead>
<tr>
<th>Study day(^a)</th>
<th>Geometric mean tick counts (min–max)</th>
<th>% Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td>2(^b)</td>
<td>23.7 (4–38)</td>
<td>0.0 (0–0)</td>
</tr>
<tr>
<td>9</td>
<td>16.7 (8–29)</td>
<td>0.0 (0–0)</td>
</tr>
<tr>
<td>16</td>
<td>15.8 (6–37)</td>
<td>0.4 (0–2)</td>
</tr>
<tr>
<td>23</td>
<td>13.9 (6–25)</td>
<td>0.6 (0–6)</td>
</tr>
<tr>
<td>30</td>
<td>20.2 (15–33)</td>
<td>1.6 (0–7)</td>
</tr>
</tbody>
</table>

\(^a\) *Live H. longicornis* were counted 48 h after treatment or infestation. Day 0 is the day of treatment.

\(^b\) Dogs were infested before treatment on Day-2.
other topical treatment does not interfere with the product efficacy.

Another product for oral administration containing spinosad includes a registered claim for *H. longicornis* tick curative treatment in Japan (Comfortis®); however, this product is not registered for use against ticks elsewhere (Beugnet and Franc, 2012; Snyder et al., 2009). Snyder et al. (2009) reported a limited, short-lasting efficacy of 50 mg/kg spinosad against new tick infestations on dogs with 67.8% efficacy at Day 9, 49.1% at Day 14, dropping to 5% at Day 30, against *Rhipicephalus sanguineus* by counting ticks 48 h after infestations. In this study, afoxolaner provided both curative and prophylactic efficacy for up to 30 days against *H. longicornis*.

Nexgard® chewable formulation containing afoxolaner represents the first oral ectoparasiticide treatment providing a month-long efficacy against *H. longicornis*, the main Japanese tick species infesting dogs.

**Conflict of interest**

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

**Acknowledgments**

The authors gratefully acknowledge the expert contributions of all collaborators from Nippon Zenyaku Kogyo Co., Ltd. (Fukushima, Japan) and Merial Limited in conducting the study to high standards.

The authors gratefully acknowledge Lenaig Halos and Frederic Beugnet, Veterinary Parasitologists, for the scientific editing of the manuscript.

**References**


Mitchell et al., 2014 (in this issue).


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