Ability of an oral formulation of afoxolaner to protect dogs from *Borrelia burgdorferi* infection transmitted by wild *Ixodes scapularis* ticks

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A B S T R A C T

A randomized, blinded, negative controlled study was conducted to determine whether treatment with afoxolaner (NexGard®, Merial, Inc.) would prevent the transmission of *Borrelia burgdorferi* to dogs by wild caught *Ixodes scapularis* ticks. Twenty healthy dogs were randomly assigned to two groups of ten dogs each. Ten dogs were treated orally on Day 0 at a dose near the minimum recommended dose of afoxolaner of 2.5 mg/kg (actual doses 2.5–3.1 mg/kg) and ten control dogs were not treated. On Day 28, each dog was infested with approximately 50 adult unfed wild caught *I. scapularis* that had a 67% *B. burgdorferi* infection rate (determined by polymerase chain reaction). On Day 33, live ticks were counted and removed. No ticks were found on treated dogs while control dogs had an average of 21.4 ticks. To detect infection, the *B. burgdorferi*-specific C6 antibody SNAP® 4Dx® test (IDEXX) was performed on serum collected before infestation (all dogs seronegative on Days -6 and 27) and on Days 48, 63, 77 and 92. The ten treated dogs remained seronegative through the end of the study (Day 92), while nine out of ten of the control dogs were infected, as demonstrated by their seroconversion to being positive for the presence of the *B. burgdorferi*-specific C6 antibody starting on Day 48. In this study, all dogs treated with NexGard® 28 days prior to challenge with wild caught *I. scapularis* ticks were protected from *B. burgdorferi* infection, while nine out of the ten untreated control dogs were infected.

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1. Introduction

Lyne disease, also called borreliosis, is a tick-transmitted disease that affects humans and dogs in North America, Europe, and Asia. It is the most frequently diagnosed vector-borne disease in humans and dogs in North America, where it is caused by the bacterial spirochete *Borrelia burgdorferi sensu stricto*, with adult and nymphal stages of *Ixodes scapularis* as principal vectors. According to Bowman et al. [1], *B. burgdorferi*-infected dogs can be found in all 48 contiguous states of the United States of America (USA).

Based on serology, *B. burgdorferi* was most frequently detected in dogs in the North-Midwest and Northeast of the USA, with an overall prevalence of 11.6% in the Northeast, and pockets of up to 61% seropositive dogs in the upper Midwest [1].

The regular use of acaricidal products may reduce the risk of tick transmission of pathogens to dogs. It is assumed that the reduction of this risk is dependent on the speed of transmission of the pathogens from the ticks to their hosts and the properties of the ectoparasiticide being used, i.e., speed of kill, ability to prevent tick attachment and/or repellent effect. Several topical acaricides have been shown to reduce the risk of transmission of vector-borne pathogens, i.e., *Babesia, Borrelia, Ehrlichia, Anaplasma*, and published studies demonstrating the utility of tick control medications for dogs have focused on their acaricidal efficacy against a broad range of ixodid tick species [2–9].

Afoxolaner belongs to a new class of insecticides-acaricides, the isoxazolines, which act systemically after dosing with an oral formulation. Afoxolaner is highly bound to plasma proteins and distributes into tissues, facilitating ingestion by hematophagous arthropods when they feed. It acts as a ligand to a specific receptor of both GABA and glutamate receptors of neuron synapses’ ion chloride channels, inducing death of fleas and ticks. The NexGard® oral formulation has been proven to provide plasma levels of afoxolaner that quickly control flea and tick infestations for a month on dogs [10]. Its protective efficacy against the transmission of *Babesia canis* by *Dermacentor reticulatus* has been demonstrated recently.

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2. Materials and methods

2.1. Animal model and treatment

The study was conducted according to Good Scientific Practices and all animal procedures were reviewed and approved by the Merial Institutional Animal Care and Use Committee (IACUC). Dogs were managed with due regard for their welfare, consistent with the US Animal Welfare Regulations (US Animal Welfare Regulations, 2008, 9 CFR). The chronology of study activities is detailed in Table 1.

This was a blinded, negative controlled clinical efficacy study using a randomized block design. Twenty healthy purpose-bred Beagle dogs, 10 male and 10 female, 8.5 to 9 months old and not previously exposed to ticks or treated with any ectoparasiticide drug were used. The dogs weighed 9 to 11.4 kg on Day -6. On Day -2, they were ranked by descending Day -6 body weight within sex. Ten blocks of 2 dogs each were formed: the 2 male dogs with the highest body weight formed Block 1, the next 2 formed Block 2, and so on, until all male dogs were allocated. The process was repeated for the female dogs. Within blocks, dog were randomly allocated to one of two treatment groups by lottery (draw-from-the-hat).

The ten dogs in Group 1 were untreated control dogs and the ten dogs in Group 2 were treated once orally on Day 0 with a combination of NexGard® (Merial, Inc.) Chews to provide a dose of afloxaner as close as possible to the minimum recommended dose of 2.5 mg/kg (actual doses ranged from 2.5 to 3.1 mg/kg, as detailed in Table 2).

On Day 28, each dog was infested with approximately 50 adult, unfed, wild-caught, I. scapularis (approximately 25 males and 25 females) known to have a 67% B. burgdorferi infection rate (see Section 2.2 next). The dogs were placed in clean individual containers (32 in long, 19 wide and 13 high) and the ticks were placed on the shoulder area of each dog. The dogs remained in the container for no longer than approximately 4 h, during which their safety and comfort were monitored and the ticks were free to roam. Sedation was allowed but was not needed.

The ticks were left on the dogs for 5 days in order to provide a complete meal and simulate the natural tick feeding behavior. On Day 33, study personnel blinded to treatment assignments examined all dogs for the presence of live ticks, which they counted and removed (Table 3). Dogs were presented for tick counting in their housing order, which was not related to their treatment group. The live or dead status of each tick was assessed before the removal of the ticks with forceps. If an attached tick appeared to be dead, it was assessed again after its removal from the animal to confirm its live/dead status. Once the entire body of the animal had been examined for ticks, the animal was combed using a flea comb to ensure that all ticks had been counted and removed.

2.2. Ticks and tick infection rates

Adult, unfed wild I. scapularis were collected in a known endemic area in southern Rhode Island (USA) between 26 October and 15 November 2014. Polymerase chain reaction (PCR) was used to determine the B. burgdorferi infection rate in the ticks based on a representative sample of 30 ticks (15 females and 15 males) selected randomly from 6 different tick storage vials. DNA was purified using a modification of the DNeasy® blood and tissue kit protocol from Qiagen (Qiagen, Inc., Valencia, CA, USA). Individual ticks were placed in 1.5 mL microcentrifuge tubes containing 180 µL of tissue lysis buffer. Ticks were crushed using a disposable 1000 µL pipette tip with its tip heat sealed. After crushing, 20 µL of proteinase K were added and samples were incubated for 2 h in a 55 °C water bath. The DNeasy tissue extraction protocol was then followed, as described by the manufacturer.

PCR amplifications to detect B. burgdorferi sensu stricto were then performed using Primers A2 [5’ GTT TGT TAA TTT CAA CTG ACC 3’] and A4 [5’ CTG CAG CITT GGA ATT CAG GCA CTT C 3’] following published methods [11], and results showed that the wild caught I. scapularis used in this study had a B. burgdorferi infection rate of 67%.

2.3. Serology

Blood (approximately 4 mL) was collected from each dog on Days -6 and 27 to confirm that they were seronegative for B. burgdorferi prior to allocation, treatment and infestation. After infestation on Day 28, blood samples were collected from all dogs as indicated in Table 1. At each time point, the blood samples were placed in individually labelled serum separator tubes. Serum was recovered, and tested using the IDEXX SNAP®4Dx® Test to detect C6 B. burgdorferi antibody, according to the manufacturer’s instructions.

2.4. Statistical methods

2.4.1. Tick counts

Efficacy with respect to live tick counts was calculated using the formula \[(C - T)/(C + T) \] × 100, where C and T are the arithmetic means of the control and treated group, respectively. The population means of the two treatment groups were compared using an F-test adjusted for the allocation blocks used to randomize the animals to the two treatment groups. This was performed by analyzing the natural logarithm of (counts + 1) using the Mixed procedure in SAS Version 9.4 with Treatment used as the only fixed effect, and Blocks used as the random effect.

2.4.2. Serology

The proportion of SNAP®4Dx® test-positive animals in the NexGard® group was compared to the proportion of positive animals in the control group at each time point using both the Pearson chi square test as well as the Cochran–Mantel–Haenszel test (CMH). The Pearson chi square test compares the difference of the propor-
tions while the CMH compares the ratio of positives in the treated to the Control group, with the latter test evaluating relative risk. Both tests were computed using the Freq procedure in SAS Version 9.4. Statistical comparisons were made using a 0.05 significance level.

3. Results

Study Day 33 tick counts and analyses are presented in Table 3. No live tick was found on any of the ten dogs treated with NexGard®, while an average of 21.4 I. scapularis were removed from the ten untreated control dogs. NexGard® treatment provided 100% percent efficacy at 5 days after tick infestations, compared to the control group, and the population means of the two groups were significantly different (p < 0.001).

The individual SNAP®4Dx® C6 B. burgdorferi antibody test results are presented in Table 4 and results of analyses of these data are presented in Table 5. All twenty dogs were seronegative prior to allocation and treatment and prior to tick infestation. The first Borrelia-seropositive dog was observed in the control group on Day 48 and the number of positive dogs in the control group increased until the end of the study. By Day 92, nine of the ten control dogs were seropositive, while none of the ten NexGard® treated dogs became seropositive at any time during the study. There was a significant difference in the expected proportion of positive between the control and the NexGard® groups on Days 63, 77, and 92 on both the Pearson chi-square test (p < 0.003) and the Cochran–Mantel–Haenszel test (p ≤ 0.004).

No clinical signs of Lyme disease were detected in any of the dogs during the daily health observations conducted at least once daily through the end of the study on Day 92.

4. Discussion

Results of this study confirm the high level of efficacy of NexGard against challenge with Ixodes scapularis ticks, as reported in other studies where the product was shown to be highly effective against this and other Ixodes species within 48 h of challenge, for up to a month [4,9]. Leaving the ticks on the dogs for 5 days after challenge allowed time for surviving ticks to take a natural and complete blood meal, which resulted in the infection of 9 out of the ten control dogs with B. burgdorferi and confirmed the validity of this transmission model.

As expected after an experimental infestation, a percentage of infesting ticks did not remain on their host, either because they were not ready to attach, or because they were groomed off by the host. Male ticks are especially susceptible to removal because they attach at a lesser rate than females. The 36–56% (average 42.8%)
Table 4
Borrelia Burgdorferi C6 antibodies SNAP® 4Dx® Results.

<table>
<thead>
<tr>
<th>Dog ID and Treatment Group Results*</th>
<th>Day -6**</th>
<th>Day 27**</th>
<th>Day 48</th>
<th>Day 63</th>
<th>Day 77</th>
<th>Day 92</th>
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<td>Number of Positive Dogs in untreated Group 1:</td>
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<tr>
<td>Number of Positive Dogs in NexGard®treated Group 2:</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* ID: Identification (n = 10 dogs per treatment group).
** Day -6 and Day 27 samples collected before the tick infestations on Day 28.
* "+" = positive; "-" = negative.

Table 5
Proportion and Analysis of the Number of Dogs Positive for Borrelia burgdorferi C6 Antibody on SNAP® 4Dx®.

<table>
<thead>
<tr>
<th>Study</th>
<th>Day</th>
<th>Number Positive*</th>
<th>P-Values*</th>
<th>Relative Risk*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>NexGard®</td>
<td>Pearson</td>
</tr>
<tr>
<td>27</td>
<td>0/10</td>
<td>0/10</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>48</td>
<td>1/10</td>
<td>0/10</td>
<td>0.305</td>
<td>0.317</td>
</tr>
<tr>
<td>63</td>
<td>6/10</td>
<td>0/10</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>77</td>
<td>8/10</td>
<td>0/10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>92</td>
<td>9/10</td>
<td>0/10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Number of dogs positive for B. burgdorferi over total number of dogs tested in Group.
* (Two-sided) probability value that the two treatment groups had different population rates.
* Pearson: Pearson chi square used to compare the difference of the two proportions.
* Relative risk test of the ratio of the treated to control proportions using the Cochran–Mantel–Haenszel test.

The absence of clinical signs of Lyme disease through the end of this study at 64 days after tick infestations (Study Day 92) is in accordance with three previous laboratory studies using a similar B. burgdorferi transmission model [5,8,12], where sporadic and episodic clinical signs of Lyme disease were observed in only one dog (lameness on days 74, 76, 90 and 92 days after tick infestation) [12]. Similarly, the majority of dogs naturally infected with B. burgdorferi do not develop clinical signs of Lyme disease [15].

Infection is a pre-requisite for disease to develop and our study focused on the demonstration of B. burgdorferi infection by detecting dogs that acquired the C6 peptide of B. burgdorferi, a specific marker of natural B. burgdorferi infection in dogs [13–18]. The IDEXX SNAP® 4Dx® test was chosen because of its extensive use in the field and its 96.7% sensitivity and 98.8% specificity for C6 B. burgdorferi antibodies [13].

The quantitative OsPA or C6 antibody tests used by Wagner and colleagues may have allowed for the detection of seroconversion at earlier time points than did the SNAP 4Dx test, but this did not seem necessary since the dogs were being monitored for seroconversion though Day 92, i.e. 64 days after tick infestations [12]. In Wagner’s study, all dogs had seroconverted to positive SNAP 4Dx by 49 days after infection [12].

Orally administered and systemically distributed insecticide/acaricidal molecules, such as afoxolaner require that ticks attach and begin feeding before being killed. Infection with pathogenic agents can be reduced by killing ticks quickly, before transmission occurs. For B. burgdorferi, transmission is thought to occur between 36 and 72 h after tick attachment to the host [19]. This study confirmed that afoxolaner was able to kill all I. scapularis ticks on treated dogs before they could transmit B. burgdorferi. A similar level of prevention was observed against Babesia canis transmission by D. reticulatus ticks in another recent study with Nexgard® [3].

In this study, a single treatment with NexGard® administered at a dose close to the minimum recommended dose of 2.5 mg/kg afoxolaner 28 days prior to tick infestation, protected dogs against the transmission of B. burgdorferi, the causative agent of Lyme disease, from naturally infected I. scapularis ticks.

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References


