

# Efficacy of Afoxolaner Alone or in Combination with Milbemycin Oxime against *Rhipicephalus sanguineus* Lato Sensu in Naturally Infested Dogs in Colombia

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## Abstract

The brown dog tick (*Rhipicephalus sanguineus* lato sensu) is the tick that most affects dogs worldwide and is therefore the main blood pathogen vector in dogs. The efficacy of afoxolaner 2.7 to 7.1 mg/kg NexGard<sup>®</sup> (group A) and afoxolaner plus milbemycin oxime 2.5 to 5.4 mg/kg and 0.5 to 1.1 mg/kg respectively NexGard Spectra<sup>®</sup> (Group B) against *R. sanguineus*, was evaluated in naturally infected sheltered dogs under high challenging conditions in four different areas of Colombia (Antioquia, Córdoba, Santander, and Meta). Tick counts (alive, dead, attached, and unattached) were performed on treated dogs, the average was calculated for the different areas to evaluate the efficacy of each treatment at six different times post-treatment (24 h, 48 h, 7 d, 14 d, 21 d, 30 d). None of the dogs showed adverse events related to the treatments. The average tick number pre-treatment was 68 in group A and 78.3 for group B indicating a strong natural infection of the dogs and their environment. Efficacy after 24 h against *R. sanguineus* was always above 90% with ≥97.4% for NexGard<sup>®</sup> and ≥93.7% for NexGard Spectra<sup>®</sup>. Consistent results were observed along all the observation periods with final efficacies (day 30) of ≥99.8% and 98.3% for NexGard<sup>®</sup> and NexGard Spectra<sup>®</sup>, respectively. In conclusion, both NexGard<sup>®</sup> and Nexgard Spectra<sup>®</sup> provided a curative effect and sustained efficacy against *Rhipicephalus sanguineus* for at least 30 days in highly contaminated shelter environments.

## Keywords

Afoxolaner, Milbemycin Oxime, *Rhipicephalus sanguineus*, Dogs, Shelter

## 1. Introduction

The control of tick infestations besides having important animal welfare implications is a method of reducing blood loss, tick feeding irritation and reducing the risk of pathogen transmission [1] [2] [3] [4]. The brown dog tick *Rhipicephalus sanguineus* lato sensu (*R. sanguineus* ls) is a constraint for animal welfare in dogs and one of the most important vectors of hemopathogens [3] [4] [5] [6]. It is the most widespread tick species in the world and is highly adapted to wet or dry climatic conditions in tropical and subtropical areas but can also thrive in temperate climates where global warming contributes to its increased presence [7].

*R. sanguineus* ls is responsible for the maintenance and transmission of multiple pathogens to domestic animals and humans [8] [9]. It is a three-host parasite and has been one of the most challenging ectoparasites, its control can be tackled based on three basic principles: the systemic control based on the use of products such as isoxazolines (afoxolaner) with a systemic effect [10] [11], the use of topic action products such as Fipronil, Pyrethrins or even organophosphorus components, all to be complemented with environmental control using S-methoprene and other metamorphosis inhibitors. Based on efficacy and ease, afoxolaner has become one of the most used strategies to control field infestations for dogs [12] [13] [14]. Up to this point in time, no tick resistance to the isoxazoline family has been reported, and the appropriate use of this new molecule will ensure them to be a long-lasting solution for tick control strategies.

According to the European Medicines Agency (EMA), to obtain a protective claim, acaricides must demonstrate the efficacy of at least 90% in the first 48 h post-treatment against existing tick infestation [15] [16] [17] [18] and >90% efficacy against new infestations until the end of the protection period to get a preventive claim. Afoxolaner is a molecule belonging to the isoxazoline family with insecticidal and acaricidal action that acts on the arthropod's  $\gamma$ -aminobutyric acid (GABA) receptor and glutamate receptors, generating an excess of neuronal stimulation and death of the arthropod [12] [13] [14].

Afoxolaner is the active ingredient of NexGard<sup>®</sup>, a highly palatable chewable tablet, providing a minimum effective dose of 2.5 mg/kg BW. It has shown effectiveness against multiple ectoparasites in dogs for at least 30 days [10] [11] [19]. Nexgard Spectra<sup>®</sup> is a chewable tablet combining afoxolaner and milbemycin oxime, providing a minimum dose of 2.5 mg/kg BW and 0.5 mg/kg BW, respectively. It allows to control fleas and ticks for a month, but also to deworm against intestinal and non-intestinal nematodes, including heartworm (*Dirofilaria immitis*) [20] [21] [22].

The aim of this field study was to assess the efficacy of afoxolaner (NexGard<sup>®</sup>, Boehringer Ingelheim) and afoxolaner plus milbemycin (Nexgard Spectra<sup>®</sup>, Boehringer Ingelheim) against *R. sanguineus* sl, in highly infested environments on naturally infested dogs in Colombia.

## 2. Materials and Methods

This study was approved under the Ethics Committee for Animal Experimenta-

tion of the University of Antioquia, act 140, 01 June 2021. The study procedures followed the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) to evaluate the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation in dogs and cats [17]. This was a multicentre study, conducted between April and July 2021 in four different regions of Colombia: Antioquia, Córdoba, Meta, and Santander, where the average temperature was 28°C, with an altitude ranging between 18 to 467 meters above sea level (as shown in **Table 1**), all of them with optimal conditions for the presence of the brown dog tick *R. sanguineus* [8].

### 2.1. Animals

From a larger shelter dog population, a total of 80 mixed breed dogs with high numbers of *R. sanguineus* ticks (confirmed taxonomically by the parasitology laboratories of the local universities) were selected and randomly assigned to the treatment groups. The inclusion criteria were clinically healthy dogs of any sex or breed, weighing not less than 2 kg and older than 8 weeks of age with natural tick infestation, with no history of ectoparasite treatment in the last 3 months. The dogs used for the study were obtained from local shelters and kept in kennels throughout the study, except for Villavicencio and Meta, where the dogs were kept in pens at the Universidad de los Llanos. Treatment and tick counts were implemented on site by trained Veterinarians at the different evaluation times: before treatment and then 24 h (T1), 48 h (T2), 7 d (T3), 14 d (T4), 21 d (T5) and 30 d (T6) after treatment.

### 2.2. Treatment

Dogs were randomly divided into two treatment groups at each testing site (A and B). Group A dogs (n = 10 per site) received afoxolaner (NexGard®) at commercial dose according to manufacturer's recommendations. Group B dogs (n = 10 per site) received afoxolaner plus milbemycin oxime (NexGard Spectra®) at commercial dose according to the label. No untreated control was kept for ethical reason, hence as previously reported by Forster *et al.*, 2021 [23], the initial pre-treatment tick count served as a baseline to allow comparison with the different post treatment counts.

### 2.3. Tick Count

The treatments were administered following the Manufacturer's recommendations at day zero to each group. Counts were performed at 24 h, 48 h, 7 d, 14 d, 21 d and 30 d to evaluate the efficacy.

Tick counts (live, dead, attached, and unattached) were performed by separating and palpating the dogs coat with the fingertips, using a medical lamp stand [17]. When a suspected tick was found, the fur was further separate, and the presence of the tick was visually confirmed without removing the tick (**Figure 1 & Figure 2**).

**Table 1.** Environmental parameters of four regions studied.

Region	Minimum temperature (°C)	Maximum temperature (°C)	Average temperature (°C)	Altitude (m.a.s.l)
Antioquia	24	36	30	150
Córdoba	28	40	34	18
Santander	23	39	31	125
Meta	29	32	30	467

**Figure 1.** Manual tick counting (Universidad de los Llanos).**Figure 2.** Manual tick counting (Universidad de los Llanos).

#### 2.4. Safety Assessment

All dogs who received either treatment were included in the safety evaluation of the product. Each of the dogs underwent a physical examination by a veterinarian prior to the initial treatment and along the trial confirming good health status.

#### 2.5. Statistical Analysis

The number of live ticks was the primary variable of the study [24]. The percentage of efficacy was calculated as the arithmetic mean (AM) of live ticks of the groups for each day pre and post treatment. Abbott's formula was used to calculate the efficacy:

$$\%Efficacy = ((Mc - Mt)/Mc) \times 100$$

where  $Mc$  is arithmetic mean of live ticks before treatment and  $Mt$  is arithmetic mean of live ticks at each post-treatment assessment time-point [25]. The analysis was performed considering statistical significance ( $p < 0.05$ ).

### 3. Results

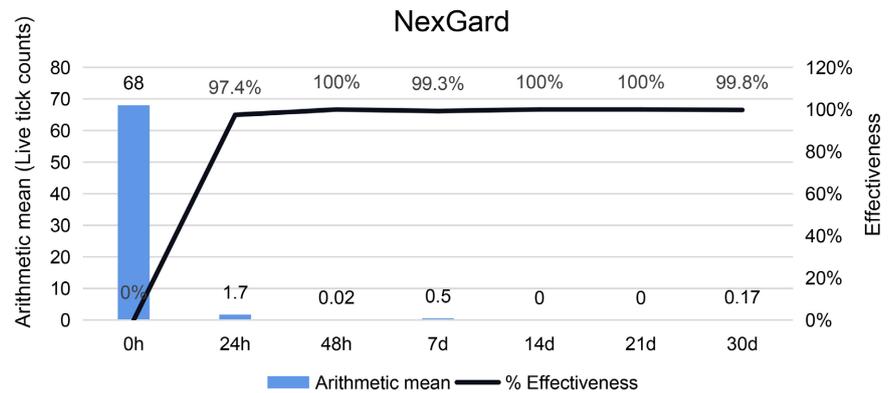
A total of 80 dogs were evaluated and treated in four different regions of Colombia where the presence of the ticks is endemic, 40 treated with NexGard and 40 treated with NexGard Spectra, 10 dogs per each group in each location (Antioquia  $n = 20$ , Córdoba  $n = 20$ , Santander  $n = 20$  and Meta  $n = 20$ ). No dogs showed any treatment-related adverse effects.

The arithmetic mean (AM) ticks and the percentage of effectiveness is summarized for all the regions in **Table 2**. The AM for group A at initial count was 68 live ticks and for group B was 78.3.

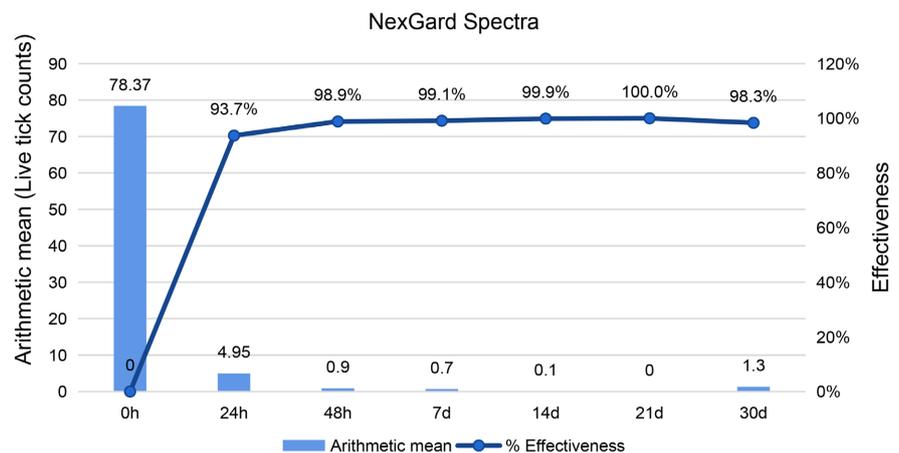
In each treatment group, all tick counts performed post-treatment were significantly different than the pre-treatment counts ( $p < 0.05$ ). The tick counts were not significantly different between the two treatment groups at each time point ( $P > 0.05$ ). At 24 h the efficacy in group A & B was 97.4%, and 93.6%, respectively, demonstrating a curative speed of kill against *R. sanguineus* within 24 h under these field conditions. At day 7 the efficacy was 99.1% and 99% respectively and was still above 98.2% at the end of the trial demonstrating a sustained preventive efficacy during at least 30 days (**Figure 3, Figure 4**).

**Table 2.** Efficacy of afoxolaner (Group A) and afoxolaner + milbemycin oxime (Group B) against *Rhipicephalus sanguineus* ls in sheltered dogs Colombia. NA = Non applicable.

Study time	Treatment group	n	Tick counts		% Effectiveness
			Range	Arithmetic mean	
Pre-treatment	A	40	3 - 385	68	NA
	B	40	4 - 900	78.3	NA
24 h	A	40	0 - 39	1.75	97.4
	B	40	0 - 152	4.95	93.7
48 h	A	40	0 - 1	0.02	99.9
	B	40	0 - 34	0.92	98.8
7 d	A	40	0 - 12	0.55	99.1
	B	40	0 - 12	0.77	99.0
14 d	A	40	0	0	100
	B	40	0 - 4	0.1	99.4
21 d	A	40	0	0	100
	B	40	0	0	100
30 d	A	40	0 - 6	0.17	99.7
	B	40	0 - 18	1.37	98.2



**Figure 3.** Efficacy and arithmetic mean (AM) of ticks in group A (Afoxolaner).



**Figure 4.** Efficacy and arithmetic mean (AM) of ticks in group B (Afoxolaner with milbemycin oxime).

#### 4. Discussion

The arithmetic means of tick counts for pre-treatment was 68 for group A, and 78.3 for group B, these numbers are higher than previously reported, suggesting high incidence conditions [25]. Albeit the pre-treatment counts were lower than a report of dogs naturally infested with *R. sanguineus* ls in Thailand that showed an AM > 800 in an arbitrarily selected group for its severe tick burden [26]. No statistically significant difference was found between the group's initial count, which allows comparison between the two groups. Previous studies conducted experimentally used weekly tick infestation with 50 ticks per dog [19] [27] [28], here the dogs were living in shelters and continuously re-infested by ticks from the environment.

The brown dog tick *R. sanguineus* ls. is the most common tick found in dogs worldwide, controlling its infestation rate on dogs is beneficial from the animal welfare standpoint and as a risk mitigation strategy for other diseases [6] [7] [29]-[35]. Overall, our results indicate that both treatments are effective at 24 h at eliminating existing *R. sanguineus* sl. infestation and then maintain the acaricidal activity for at least 1 month against the natural reinfestations [28].

For this trial, a comparison with a non-treated infested control group was not available under field conditions due to animal welfare considerations. The elevated tick counts at pre-treatment indicated high tick burdens in the shelters where the dogs were kept confirming the efficacy of afoxolaner alone or in combination with milbemycin to control the parasite load. In conclusion, Nexgard and Nexgard spectra were demonstrated safe and efficacious for the treatment and control of *Rhipicephalus sanguineus* lato sensu, in naturally infested dogs in Colombia for at least 30 days.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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