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Short Communication

Comparative efficacy of two oral treatments for dogs containing either afoxolaner or fluralaner against *Rhipicephalus sanguineus* sensu lato and *Dermacentor reticulatus*

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ABSTRACT

The present study compares the efficacy of two recent oral ectoparasiticides containing isoxazolines (NexGard[®], containing afoxolaner and administered at a monthly regimen, and Bravecto[™] containing fluralaner and administered at a tri-monthly regimen) against *Rhipicephalus sanguineus* sensu lato and *Dermacentor reticulatus* ticks on dogs.

24 dogs were randomly allocated to untreated control, NexGard[®] treated, and Bravecto[™] treated groups. The treatments were administered on Days 0, 28 and 56 for afoxolaner and on Day 0 for fluralaner. Tick infestations were performed weekly with 50 unfed adult ticks per each species on each dog from Days 30 to 84 (with the exception of *R. sanguineus* on Day 63). Ticks were counted at 24 h post-infestation.

The dogs from both treated groups had statistically significantly ($p < 0.05$) less *R. sanguineus* and *D. reticulatus* ticks compared to the untreated dogs on all assessment days. Percent efficacy against *R. sanguineus* ranged from 86.4% to 99.5% at 24 h post-infestation for NexGard[®] and from 65.7% to 100% for Bravecto[™]. Statistically significantly ($p < 0.05$) less *R. sanguineus* ticks were recorded for NexGard[®] treated dogs compared to Bravecto[™] treated dogs on Day 78. Percent efficacy against *D. reticulatus* ranged from 85.2% to 99.6% at 24 h post-infestation for NexGard[®] and from 63.4% to 99.1% for Bravecto[™]. Statistically significantly ($p < 0.05$) less *D. reticulatus* ticks were recorded for NexGard[®] treated dogs compared to Bravecto[™] treated dogs on Days 71, 78 and 85.

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1. Introduction

Ticks of the genus *Dermacentor* and *Rhipicephalus* are of the most important vectors of severe canine diseases (Chomel, 2011; Halos et al., 2013; Otranto et al., 2009). The geographic distribution of both *Rhipicephalus sanguineus* sensu lato and *Dermacentor reticulatus* tick species is

expanding in Europe as a result of climate changes and increasing movements of people travelling abroad with their pets (Beugnet and Chalvet-Monfray, 2013; Dantas-Torres et al., 2013). According to the standard determined by pharmaceutical regulation worldwide, an anti-tick product for dogs should provide a minimal activity of 90% acaricidal efficacy, characterized by a reduction in tick counts 48 h after treatment and subsequent re-infestation (Beugnet and Franc, 2012; EMEA, 2000; Halos et al., 2012; Marchiondo et al., 2013). Recently, oral systemic flea and tick control formulations have been developed. Both

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afoxolaner and fluralaner are new insecticide-acaricide molecules from the isoxazoline family that act on the insect γ -aminobutyric acid receptor (GABA) and glutamate receptors, resulting in excess neuronal stimulation and death of the arthropod (Gassel et al., 2014; Lahm et al., 2013; Shoop et al., 2014). Afoxolaner is the active ingredient of NexGard® (Shoop et al., 2014), and fluralaner is the active ingredient of Bravecto™ (Rohdich et al., 2014). Due to their pharmacokinetic properties and the minimum dose administered, 2.5 mg/kg of afoxolaner and 25 mg/kg of fluralaner, respectively, they provide long-lasting insecticidal and acaricidal activity against fleas and ticks (Letendre et al., 2014; Kilp et al., 2014). NexGard® administered to dogs kills ticks within 48 h after infestation for 4 weeks (EMA, 2014a, b; Dumont et al., 2014; Kunkle et al., 2014), and Bravecto™ kills ticks within 48 h after infestation for 8–12 weeks, according to tick species (EMA, 2014a,b). Because of the systemic mode of action of the products, ticks must attach and start ingesting material before being killed. It can be hypothesized that ticks could be killed sooner than 48 h after infestation in relation to the plasma concentration of the actives (Halos et al., 2014). The aim of this study was to provide a comparative assessment of killing efficacy at 24 h against ticks during a 12 weeks period for a single Bravecto™ administration or 3 successive monthly administrations of NexGard®, according to their respective labelling in Europe.

2. Materials and methods

The study was a parallel group design, randomized, single centre, blinded, controlled, study conducted in respect of the Good Clinical Practices as described in International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guideline GL9 (EMA, 2000). It included three groups of 8 dogs each: untreated dogs, NexGard® treated, and Bravecto™ treated.

The 24 dogs included were males and females of mixed breeds, older than 6 months, with no restriction related to hair length, and weighing between 12 and 27.7 kg. They were clinically healthy, and they had not been treated with a long acting topical or systemic acaricide/insecticide during the 12 weeks preceding Day 0. The dogs were individually housed in cages.

Treatments were administered in accordance with the European registration labels of NexGard® and Bravecto™ on Days 0, and again on Days 28 and 56 for NexGard®. The dogs were fed immediately after administration according to Bravecto™ labelling.

Laboratory-bred strains of *R. sanguineus* sensu lato (strain originally collected in France and laboratory bred) and *D. reticulatus* (strain originally collected in France and laboratory bred) were used for the infestations. The adult ticks were unfed, at least 3 months old and had a balanced sex ratio (50% female/50% male). Each dog was artificially infested with 50 ticks per species (sex ratio 50/50) on each infestation day (Days 28, 35, 42, 49, 56, 63, 70, 77 and 84). On Days 28 and 56, NexGard® treatment was applied at the time of tick challenge. Ticks were directly deposited on the mid-line of the dogs.

Tick counting was as close as possible to the specified target time of 24 h \pm 2. Ticks were assessed as attached or free, dead or alive, and they were removed from the dogs 48 h after each infestation.

Efficacy against ticks was calculated for the treated groups at each assessment day in accordance with WAAVP guidelines, using the Abbott's formula (Marchiondo et al., 2013).

$$\text{Efficacy(\%)} = 100 \times \frac{M_c - M_t}{M_c}$$

where M_c = arithmetic mean of live (attached or free) ticks on the negative control group (group 1) and M_t = arithmetic mean of live (attached or free) ticks on the Treatment-administration groups (groups 2 or 3).

The groups were compared by a one-way ANOVA with an administration effect on the untransformed live tick counts.

3. Results

The arithmetic mean tick count for the negative control group ranged from 19.5 to 39.8 for *Dermacentor* and from 26.6 to 40.1 for *Rhipicephalus*, indicating viable tick challenges on all assessment days (Table 1). The treated groups had statistically significantly ($p < 0.05$) less *D. reticulatus* and *R. sanguineus* ticks compared to the untreated control group on all assessments days. Statistically significantly ($p < 0.05$) less *D. reticulatus* ticks were recorded for NexGard® treated dogs compared to Bravecto™ treated dogs at 24 h post-infestation on Days 71, 78 and 85. The difference was significant on Day 78 for *R. sanguineus* (Table 1). No adverse events related to any of the treatments were observed.

4. Discussion

Isoxazolines represent a new class of ectoparasiticides acting systemically after oral administration (Shoop et al., 2014; Gassel et al., 2014). Duration of effect is linked to the dose of active administered, the peak plasma levels achieved after ingestion, protein binding, and the drug's terminal plasma half-life of 12–15 days for fluralaner formulation (Kilp et al., 2014); and 15.5 (± 7.8) days for afoxolaner formulation (Letendre et al., 2014). The first tick infestations were conducted on Day 28 based on the hypothesis that the fluralaner concentration would be still high enough to provide good efficacy at 24 h counts. This was confirmed by the 98.7% and 100% of observed efficacies at Day 29 on *Dermacentor* and *Rhipicephalus*, respectively.

Thereafter, the fluralaner efficacy observed at 24 h significantly decreased during the 3rd month. In contrast the monthly administration of afoxolaner provided constant efficacy against both species of ticks even when evaluated at 24 h after challenge. It indicates that tick death may be linked to the duration of exposure to the actives. A longer exposure is needed when the plasma concentration of the acaricidal compound is lower, leading to a slower speed of kill. The present study demonstrates that a monthly administration of afoxolaner offers a more homogeneous and constant protection compared to a single treatment

Table 1
Efficacy against *Dermacentor reticulatus* and *Rhipicephalus sanguineus* sensu lato ticks observed at 24 h (arithmetic means).

| Days of infestation | <i>Dermacentor reticulatus</i> Arithmetic mean of tick counts at 24 h | | | <i>Rhipicephalus sanguineus</i> sensu lato Arithmetic mean of tick counts at 24 h | | |
|---------------------|--|--|---|--|--|---|
| | Control group | NexGard® treated group (%efficacy) | Bravecto™ treated group (%efficacy) | Control group | NexGard® treated group (%efficacy) | Bravecto™ treated group (%efficacy) |
| Day 28 | 19.5 | 0.1* (99.4) | 0.3* (98.7) | 26.6 | 0.1* (99.5) | 0.0* (100.0) |
| Day 35 | 39.8 | 0.4* (99.1) | 0.4* (99.1) | 40.1 | 0.8* (98.1) | 0.5* (98.8) |
| Day 42 | 34.1 | 2.0* (94.1) | 2.1* (93.8) | 35.8 | 1.0* (97.2) | 1.5* (95.8) |
| Day 49 | 30.3 | 1.4* (95.5) | 0.9* (97.1) | 33.6 | 2.1* (93.7) | 1.9* (94.4) |
| Day 56 | 32.4 | 0.1* (99.6) | 2.9* (91.1) | 36.1 | 0.9* (97.6) | 2.8* (92.4) |
| Day 63 | 37.1 | 0.4* (99.0) | 7.3* (80.5) | NA | NA | NA |
| Day 70 | 34.0 | 1.4*¹ (96.0) | 8.4*¹ (75.4) | 34.5 | 2.8* (92.0) | 8.6* (75.0) |
| Day 77 | 33.6 | 1.9*¹ (94.4) | 11.9*¹ (64.7) | 33.5 | 2.1*¹ (93.7) | 11.5*¹ (65.7) |
| Day 84 | 30.4 | 4.5*¹ (85.2) | 11.1*¹ (63.4) | 34.1 | 4.6* (86.4%) | 9.6* (71.8%) |

*p-Value from control <0.0001. p-Value: one-way ANOVA with a treatment effect.

¹(bold): statistically significantly differences ($p < 0.05$) were recorded between treated groups.

%Efficacy = $100 \times (M_c - M_t) / M_c$, where M_c = arithmetic mean of live (attached of free) ticks on the negative control group and M_t = arithmetic mean of live (attached of free) ticks on the Treatment-administration groups.

with fluralaner. Similar conclusions were recently obtained in regard to flea efficacy (Beugnet et al., 2015). Assessing anti-tick efficacy at earlier time-points than the classical 48 h provides an idea of the potential to decrease the risk of pathogen transmission, even if these molecules act by systemic way. It has been recently demonstrated with afoxolaner preventing the transmission of *Babesia canis* by infected ticks (Beugnet et al., 2014).

Conflict of interest

This clinical study was funded by Merial S.A.S., 29 avenue Tony Garnier, 69007 Lyon of which Frederic Beugnet and Lénaïg Halos are employees.

ClinVet, of which Julian Liebenberg is employee, is an independent, South African, Contract Research Organization contracted to conduct the study.

All authors voluntarily publish this article and have no personal interest in these studies other than publishing the scientific findings that they have been involved in via planning, initiating, monitoring and conducting the investigations and analyzing the results.

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NexGard® is a registered trademark of Merial and Bravecto™ is a trademark of Merck. All other marks are the property of their respective owners.

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