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Comparative speed of efficacy against *Ctenocephalides felis* of two oral treatments for dogs containing either afoxolaner or fluralaner

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ABSTRACT

A study was designed to compare the efficacy of NexGard® and Bravecto™, 2 recently introduced oral ectoparasiticides containing isoxazolines, against fleas (*Ctenocephalides felis*) on dogs. Twenty-four healthy dogs, weighing 9.2 kg to 28.6 kg, were included in this parallel group design, randomized, and controlled efficacy study. On Day −1, the 24 dogs were allocated to 3 study groups: untreated control; Nexgard® treated and Bravecto™ treated. The treatments were administered on Days 0, 28 and 56 for Nexgard® (labelled for monthly administration), and once on Day 0 for Bravecto™ (labelled for a 12 week use). Flea infestations were performed weekly with 100 adult unfed *C. felis* on each dog from Days 42 to 84. Fleas were counted and re-applied at 6 and 12 h post-infestation and removed and counted 24 h post-infestation. The arithmetic mean flea count for the untreated group ranged from 62.9 to 77.6 at 24 h post-infestation, indicating vigorous flea challenges on all assessment days. Both the Nexgard® and Bravecto™ treated groups had statistically significantly ($p < 0.05$) less fleas compared to the untreated group on all assessment time points and days. Significantly fewer fleas were recorded for NexGard® treated dogs compared to Bravecto™ treated dogs at 6 h post-infestation on Day 56, 63, 70, 77 and 84 and at 12 h post-infestation on Days 70 and 84. No statistically significant ($p < 0.05$) differences were recorded between the treated groups at 24 h post-infestation. Efficacies recorded 6 h post-infestation for Nexgard® ranged from 62.8% (Day 49) to 97.3% (Day 56), and efficacies ranged from 94.1% (Day 49) to 100% (Days 42, 56, 70 and 84) at 12 h post-infestation. Efficacies recorded for Bravecto™ ranged from 45.1% (Day 84) to 97.8% (Day 42) at 6 h post-infestation, and from 64.7% (Day 84) to 100% (Days 42 and 56) at 12 h post-infestation. Efficacies observed at 24 h were 100% for both products during the study except 99.6% on Day 84 for Bravecto™.

1. Introduction

Fleas represent one of the most prevalent parasites in domestic carnivores (Beugnet et al., 2014a,b). In addition to causing discomfort to pets and their owners, *C. felis* is primarily responsible for flea bite allergy dermatitis in both dogs and cats (Dryden and Rust, 1984). Cat fleas are also vectors of several pathogens including *Dipylidium caninum*, *Rickettsia felis*, and *Bartonella henselae* (Otranto et al., 2009a, 2009b).

Although the use of insecticides such as fipronil, imidacloprid, selamectin, and spinosad have revolutionized

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flea control in the recent years, treatment and prevention of cat flea infestations remain a major concern for pet owners and veterinarians (Beugnet and Franc, 2012; Halos et al., 2014). Licensed products must reach standards of efficacy determined by pharmaceutical regulatory authorities worldwide (e.g. European Medicine Agency (EMA) in Europe, Environmental Protection Agency (EPA) or Food and Drug Agency (FDA) in the USA) (EMEA, 2000; Marchiondo et al., 2013). To meet EMA guidelines, the efficacy of a drug against fleas must reach at least 95%, 48 h post infestation for a given time-point in several controlled standard studies.

Several criteria are judged important for both veterinarians and pet owners: the ease of treatment, the duration of efficacy, and the speed of kill (Beugnet and Franc, 2012; Halos et al., 2014; Otranto and Wall, 2008). The convenience of administration is a key factor for treatment compliance. In that aspect, topical spot-on formulations represented a major advance in the 1990s. More recently, chewable oral flea or flea and tick control formulations have been developed. Depending on the palatability, the dogs may spontaneously consume the chewable tablet which contains the insecticide-acaricide. Both 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, inhibiting GABA and glutamate-regulated uptake of chloride ions 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® (Otranto, 2014), and fluralaner is the active ingredient of Bravecto™ (Rohdich et al., 2014). The two formulations are highly palatable to enhance compliance of treatments administered by dog owners. 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 has been shown to kill 100% of fleas within 24 h after infestation for 5 weeks (Hunter et al., 2014), and Bravecto™ has been demonstrated to kill 100% of fleas within 48 h after infestation for 12 weeks, according to the U.S. product label (Bravecto prescribing information. Summit, NJ, Merck Animal Health, 2014).

Speed of kill is also an important criterion for assessing a flea control product, because the more quickly fleas are killed the less likely a pet owner is to observe them on the pet. Speed of kill also influences flea egg production, and faster speed of kill therefore results in less flea egg contamination of the environment (Beugnet et al., 2014a,b; Hunter et al., 2014; Williams et al., 2014). Both NexGard® and Bravecto™ have demonstrated a flea efficacy above the threshold of 95% starting as early as 4–8 h after flea challenges (Beugnet et al., 2014a,b; Hunter et al., 2014). Based on the published data on the duration of efficacy, as well as the potential speed of kill, the aim of this study was to compare the speed of kill against fleas between one Bravecto™ administration and 3 successive 28 day administrations of NexGard®, according to their respective labelling in Europe (Beugnet et al., 2014a,b; Hunter et al., 2014; Rohdich et al., 2014).

2. Materials and methods

2.1. Study design

The study was a parallel group design, randomized, single centre, blinded, controlled, efficacy study. In order to prevent any bias, the treatments were not administered to dogs by an individual involved in performing the post-administration assessments and observations. The groups were coded to blind the staff performing post-administration observations and assessments. The study was conducted on three groups of 8 dogs each. The study was authorized by both Merial and ClinVet ethical committees, and was 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 (EMEA, 2000). Group 1 dogs remained untreated while group 2 dogs were treated orally with NexGard® and group 3 dogs with Bravecto™.

The design of the study is shown in Table 1. The study followed a randomized block design. The 24 dogs included were ranked within sex in descending order of individual pre-administration flea counts and subsequently blocked into eight replicates of three dogs each. Within blocks, dogs were randomly allocated to three coded groups. The coded groups were randomly assigned to the treatments by a non-blinded person external from the study staff.

2.2. Animals

In order to assess the individual susceptibility to flea bites, 27 dogs were originally challenged with fleas and the 3 dogs with the lowest flea infestations were excluded. The 24 included dogs were males and females of mixed breeds, older than 6 months, with no restriction hair length, and weighing between 9.2 and 28.6 kg. They were

Table 1
Study design.

Acclimatation	Flea infestations	Ranking and allocation to the 3 groups	Treatments	Assessments at 6 and 12 h – in situ flea counts	Assessments at 24 h – flea counts and removal
Days −14 to −1	Days −7, 42, 49, 56, 63, 70, 77 and 84	Day −2 (±1 day)	NexGard®: Day 0, 28 and 56 (Group 2) Bravecto™: Day 0 (Group 3)	Days 42,49, 56, 63, 70, 77 and 84	Days −5, 43, 50, 57, 64, 71, 78 and 85

clinically healthy as confirmed by a veterinarian on Day −14, not pregnant, and they had not been treated with a long acting topical or systemic acaricide/insecticide during the 12 weeks preceding Day 0 (day of administration).

The dogs were individually housed in boxes which consisted of a 1.69 m × 0.7 m enclosed sleeping area, with heating provided as necessary, and an outside run of 1.69 m × 3.0 m. A roof covers the kennels and the dogs were not exposed to rain. Dogs were provided with chew toys on a weekly basis.

The animals were fed according to the food manufacturer's recommendation.

2.3. Treatment

Treatments were administered in accordance with the European registration labels of NexGard® and Bravecto™ on Days 0, and again on Days 28 and 56 with NexGard® (Table 2). On Day 56, the flea infestation was performed after the administration of NexGard®. The dogs were fed immediately after administration according to Bravecto™ labelling. All the animals were observed daily (from Days −14 to 85) for general health conditions and clinical signs of any adverse effects to the treatment.

2.4. Flea infestations and counts

A laboratory bred strain (derived from a European isolate) of *C. felis* was used for all infestations. The fleas were unfed and of mixed sex. Each dog was infested with 100 fleas (sex ratio 50:50) on the days as indicated in Table 1.

Flea counts were conducted by combing at specified times after infestation: 6 h (±30 min), 12 h (±1 h) or 24 h (±2 h) post-infestation.

A fine-toothed flea comb was used to recover fleas present in the animal's fur. The method of combing was by several strokes of the comb in each area of the animal, each time moving in the same direction, following the pattern of the hair coat. Movement, from one part of the animal's fur to the next, was via strokes overlapping each other, so that no area of fur was missed. The examined areas were: outside hind legs, including feet; tail and anal areas; lateral area, not including shoulders; abdominal area, from chest to inside hind legs; fore legs and shoulders, including feet; all neck and head areas; and dorsal strip from shoulder blades to base of tail. After completion of the combing procedure for all body areas, the whole procedure was repeated once more so that all areas were combed at least twice. During the 6 h and 12 h post-infestation counts, the live fleas were placed back on the dog and they were only finally removed during the 24 h post-infestation counts.

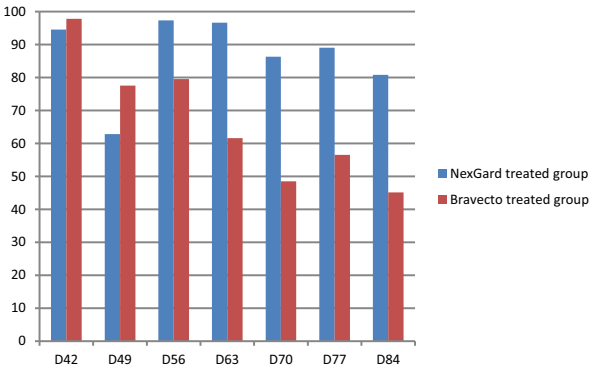

Fig. 1. Comparative efficacy observed during the study at 6 h counts post-infestation.

2.5. Statistical analysis

Efficacy against fleas was calculated for the treated groups at each assessment day, in accordance to 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 fleas on the negative control group (group 1)

M_t = arithmetic mean of live fleas on the Treatment – administration groups (groups 2 or 3)

The statistical unit is the specific group if central values of a group are compared. In other cases, the statistical unit is the individual animal.

The groups were compared at each time point by a one-way ANOVA with an administration effect on the untransformed live flea counts. SAS Version 9.3 was used for all the statistical analyses.

3. Results

No adverse events related to any of the treatments were observed.

Arithmetic flea counts and efficacies based on the various time points and days for all groups are summarized in Table 3 and Fig. 1. The arithmetic mean flea count for the

Table 2
NexGard® and Bravecto™ posology.

Group	Sample size	Product	Active ingredients	Dose administered
2	8	NEXGARD	Afoxolaner	3 g chew (68 mg afoxolaner) for dogs >10–25 kg 6 g chew (136 mg afoxolaner) for dogs >25–50 kg
3	8	BRAVECTO	Fluralaner	250 mg chewable for dogs > 4.5–10 kg 500 mg chewable for dogs > 10–20 kg 1000 mg chewable for dogs > 20–40 kg

Table 3
Efficacy of NexGard® and Bravecto™ against fleas during the 12 week period of the study, based on Arithmetic means.

Day & time of flea count Group	Untreated	Mean (Eff%) NexGard® treated	Mean (Eff%) Bravecto™ treated	*p*-value[a]
Day 42				
6 h	72.8	4.0 (94.5%)	1.6 (97.8%)	NS
12 h	70.9	0.0 (100.0%)	0.0 (100.0%)	NS
24 h	66.5	0.0 (100.0%)	0.0 (100.0%)	NS
Day 49				
6 h	90.6	33.8 (62.8%)	20.4 (77.5%)	NS
12 h	82.4	4.9 (94.1%)	1.8 (97.9%)	NS
24 h	77.6	0.0 (100.0%)	0.0 (100.0%)	NS
Day 56				
6 h	**77.4**	**2.1 (97.3%)**	**15.9 (79.5%)**	**0.0384**
12 h	76.8	0.0 (100.0%)	0.0 (100.0%)	NS
24 h	74.6	0.0 (100.0%)	0.0 (100.0%)	NS
Day 63				
6 h	**85.4**	**2.9 (96.6%)**	**32.8 (61.6%)**	**0.002**
12 h	75.1	0.3 (99.7%)	3.5 (95.3%)	NS
24 h	68.3	0.0 (100.0%)	0.0 (100.0%)	NS
Day 70				
6 h	**85.1**	**11.6 (86.3%)**	**43.9 (48.5%)**	**0.0059**
12 h	**76.0**	**0.0 (100.0%)**	**8.9 (88.3%)**	**0.0486**
24 h	62.9	0.0 (100.0%)	0.0 (100.0%)	NS
Day 77				
6 h	**89.9**	**9.9 (89.0%)**	**39.1 (56.5%)**	**0.007**
12 h	77.8	0.1 (99.8%)	11.6 (85.0%)	NS (0.055)
24 h	69.0	0.0 (100.0%)	0.0 (100.0%)	NS
Day 84				
6 h	**84.8**	**16.3 (80.8%)**	**46.5 (45.1%)**	**0.0065**
12 h	**73.3**	**0.0 (100.0%)**	**25.9 (64.7%)**	**0.0025**
24 h	65.6	0.0 (100.0%)	0.3 (99.6%)	NS

NS: not significant (p-value > 0.05). Bold lines: significant difference between flea counts in the two treated groups.
[a] One-way ANOVA with an administration effect on the untransformed live flea counts comparing the two treated groups.

negative untreated group ranged from 62.9 to 77.6 at 24 h, indicating robust flea challenges on all assessment days.

Both the afoxolaner and fluralaner treated dogs had statistically significantly ($p < 0.05$) less fleas compared to the untreated control dogs at all assessment time points on all days of evaluation.

Statistically significantly ($p < 0.05$) fewer fleas were recorded for the Nexgard® treated dogs compared to the Bravecto™ treated dogs at 6 h post-infestation on Day 56, 63, 70, 77 and 84 and at 12 h post-infestation on Days 70 and 84.

No statistically significant ($p < 0.05$) differences were recorded between the flea counts recorded for the treated groups at 24 h post-infestation.

Efficacies recorded at 6 h post-infestation for NexGard® ranged from 62.8% (Day 49) to 97.3% (Day 56), and at 12 h post-infestation ranged from 94.1% (Day 49) to 100% (Days 42, 56, 70 and 84).

Efficacies recorded at 6 h post-infestation for Bravecto™ ranged from 45.1% (Day 84) to 97.8% (Day 42), and at 12 h post-infestation ranged from 64.7% (Day 84) to 100% (Days 42 and 56).

4. Discussion

Insecticidal and acaricidal isoxazolines represent a new class of ectoparasiticides acting systemically after oral administration (Shoop et al., 2014; Gassel et al., 2014). Based on their pharmacokinetic properties, a long-lasting effect is observed. It is directly linked to the dose of active administered, the peak plasma levels achieved after ingestion, the binding on plasmatic proteins, and the drug's terminal plasma half-life of 12–15 days for fluralaner formulation and 15.5 (±7.8) days for afoxolaner formulation (Kilp et al., 2014; Letendre et al., 2014). We hypothesize that the speed of kill of fleas should be correlated to the number of flea bites and amount of blood ingested by fleas. Bravecto™, dosed at 25 mg/kg minimum, showed 100% efficacy against fleas counted at 48 h after infestation 12 weeks after treatment (Bravecto™ prescribing information. Summit, NJ, Merck Animal Health, 2014). NexGard®, dosed at 2.5 mg/kg minimum, provided 100% efficacy against fleas 24 h after infestations at all timepoints in this study, which is comparable to previously published results (Hunter et al., 2014).

Assessments 24 h after infestation can provide an indication of killing fleas before most will lay eggs, therefore impacting the potential for re-infestation (Dryden, 1989). Assessments at 48 h after infestation are typically used for regulatory purposes (Marchiondo et al., 2013). Earlier assessments provide an indication of how fast fleas will be eliminated, therefore reducing the risk of bites.

A decrease in the speed of kill over time is typically observed with systemic flea control products during the time when plasma concentrations of active ingredient decline due to metabolism and elimination. This was observed in this study, with significant differences in flea counts between the 2 treated groups at 6 h post-challenges from Day 56 to Day 84. During this time frame, fluralaner plasma levels were the result of dosing on Day 0 and were

declining according to its terminal half-life (Letendre et al., 2014; Kilp et al., 2014). The decrease in the speed of kill was recently studied by Taenzler et al. who demonstrated that after flea re-infestations in weeks 4, 8 and 12, the efficacy of fluralaner at 4 h was 96.8, 91.4, and 33.5%, respectively (Taenzler et al., 2014).

Afoxolaner plasma levels were the result of dosing on study Day 56. Dosing NexGard® every 28 days provided significantly greater efficacy at 6 h – post-infestation than one dose of Bravecto™ during its last 4 week dosing period.

In this study, the first flea counts were initiated at Day 42 as it was assumed by the authors that the efficacy would stay high for at least for 6 weeks in relation to a high plasma level of fluralaner (Gassel et al., 2014). This was somehow confirmed by the efficacies observed on Day 42 at 6 h which was 97.8% for Bravecto™. The first point where a difference was significant occurred at 6 h counts on Day 56.

A reduction in the prophylactic speed of kill in the last weeks (weeks 9 to 12) may have clinical consequences as both dog owners and veterinarians may see fleas on the treated dogs until they die. The number of flea bites may also be more important if fleas take of a longer time to die.

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.

Note from the authors

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

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