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Insecticidal efficacy of afoxolaner against *Stomoxys calcitrans* (Diptera: Muscidae) in dogs

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ABSTRACT

The insecticidal activity of oral afoxolaner (NexGard®) against stable flies *Stomoxys calcitrans* (Diptera: Muscidae) that had fed on dogs was evaluated in a blinded, randomised, and negative controlled efficacy study. The efficacy assessments were based on survival rates of fed flies after challenges to treated dogs. For a challenge, each dog was exposed to 50 unfed *S. calcitrans* for 30 minutes, after which time live fed flies were collected and incubated in an insectarium for viability assessment after 48, 72 and 96 hours. Fourteen dogs were randomly allocated to an untreated control group and an afoxolaner-treated group of seven dogs each. NexGard® was administered on Day 0 per label instructions to the treated group. All dogs were challenged on Days 1, 7, 14, 21 and 28. Efficacy was calculated by comparison of the proportion of incubated live fed flies for each individual after their related 30-min challenges in the control and treated groups after 48, 72 and 96 hours of incubation. A significant afoxolaner activity against *S. calcitrans* was demonstrated, with efficacy at 96 hours after blood-feeding ranging from 76.4 to 98.5% through Day 28.

1. Introduction

Stable flies (family Muscidae, genus *Stomoxys*), are obligate blood-sucking dipterans and are distributed worldwide (Bowman, 2009; Russel et al., 2013). Being the most common blood-feeding flies around farm animals, stable flies are considered as an important economic pest of livestock (Campbell et al., 2001; Elkan et al., 2009; Lienard et al., 2011) and can also affect other warm-blooded species, including domestic animals (Bowman, 2009; Russel et al., 2013) and humans (Janovy and Roberts, 2000; Dominghetti et al., 2015). Both male and female stable flies are hematophagous and bite during daytime. They are more abundant in the vicinity of livestock and are significantly more active in warm latitudes or during the warm seasons in temperate regions (Muenworn et al., 2010; Sholwer and Osbrink, 2015). Females lay eggs on faeces or soiled and decayed material, such as damp hey, piles of lawn clippings, and shoreline deposits of seaweeds, after three or more blood meals, and up to three ovipositions can be observed in one day (Salem et al., 2012). Larval and nymphal stages develop in the environment in one to several weeks depending on the temperature and humidity, until emergence of blood-sucking adult forms (Bowman, 2009; Russel et al., 2013).

In dogs, the bites of stable flies can cause continuous irritation and restlessness during the daylight hours. They namely attack ears and the dorsal part of the muzzle. Lesions may range from pinpoint hemorrhages to necrotic dermatitis (Yeruham and Braverman, 1995). Dogs housed outdoors, such as kennels, and hunting dogs, are particularly exposed to stable flies' attacks (Urban and Broce, 1998).

Stable flies may be responsible for inter-host mechanical transmission of pathogens present in the blood and on the skin. When injecting saliva prior to blood-sucking, they may inoculate infected material remaining on their mouthparts from a previous host (Johnson et al., 2010; Lienard et al., 2012). Equine infectious anemia, African swine fever, West Nile, and Rift Valley viruses, bovine papillomavirus, as well as rickettsial bacteria (*Anaplasma* spp., *Coxiella* spp.), and protozoans (*Trypanosoma* spp., *Besnoitia* spp.) are known to be transmitted by *Stomoxys* (Mihok et al., 1995; Scoles et al., 2005; Mohammed et al., 2010; Turrell et al., 2010; Doyle et al., 2011; Haspeslagh et al., 2018; Sharif et al., 2019). *Stomoxys* flies are also known to act as an intermediate host of the helminth *Habronema microstoma* which infect horses, and may be involved in the transmission of some species of *Onchocerca* and *Dirofilaria* (Baldacchino et al., 2013).

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Afoxolaner is a systemic insecticidal and acaricidal compound from the isoxazoline group, available as an oral dog product (NexGard® and NexGard® Spectra) and indicated for the treatment and control of flea, tick and mite infestations (European Medicines Agency, 2021). After oral administration, afoxolaner is rapidly absorbed with plasma peak levels observed between 2 and 4 hours after administration and is highly bound to plasma proteins, therefore acting through a systemic pathway on hematophagous arthropods (Letendre et al., 2014, 2017). Afoxolaner acts by inhibition of a specific receptor on gamma-aminobutyric acid (GABA)-gated chloride ion channels, resulting in uncontrolled activity of the central nervous system and death of the arthropod (Ozoe et al., 2010; Shoop et al., 2014). The insecticidal activity of afoxolaner has already been described against the flying insects *Aedes aegypti* (Liebenberg et al., 2017) and *Phlebotomus perniciosus* (Perier et al., 2019), and against *Cimex lectularius* (Beugnet et al., 2021).

This manuscript describes a study designed to assess the afoxolaner insecticidal activity against *Stomoxys calcitrans* after feeding on treated dogs.

2. Materials and methods

This was a single center, blinded, negative controlled efficacy study, using a randomized block design, and conducted in accordance with VICH GL 9 Good Clinical Practices principles.

Fourteen purpose-bred healthy adult dogs were included in the study and are described in Table 1. Dogs had not been exposed to any acaricide/insecticide compound over the 12 weeks preceding the study. Dogs were acclimatized to the study conditions for 2 weeks before afoxolaner administration and were observed daily for general health, adverse reactions, and reactions to *S. calcitrans* bites.

The experimental model was based on weekly challenges to *S. calcitrans*, followed by the incubation of live fed flies in an insectarium and survival assessments. For a challenge, each dog was sedated, placed in an individual insect proof net-equipped enclosure, and then exposed to 50 ± 5 unfed *S. calcitrans*. After 30 minutes of exposure, the flies were collected, classified, and counted per individual dog, the sedations reversed, and the dogs returned to their housing. The flies were classified as fed or unfed, and live or dead (moribund flies were considered live). The feeding status was determined visually by the swelling of the abdomen. All collected live fed flies were placed in a container labelled with their corresponding dog identification, and incubated in the same insectarium at 26 (±4) °C with an access to a 10% sucrose solution. After 48, 72 and 96 hours of incubation, the flies were evaluated for live or dead status.

During the acclimatization period, an initial *S. calcitrans* challenge was conducted to evaluate the suitability of each dog for the experimental exposure and for random allocation of the animals to the study groups. The allocation was based on live fed stable fly counts 48 hours after a 30-min exposure, and seven dogs were assigned to each group. The mean/median and minimum/maximum numbers of live fed stable flies after 48 hours of incubation were 29.9/28.0 and 24/37 in the control group, and 29.9/28.0 and 23/41 in the treated group, respectively.

On Day 0, all dogs from the afoxolaner group were treated orally with a NexGard® chew, in accordance with the label instructions. Dogs assigned to the untreated control group were sham-dosed (Table 1).

The insecticidal activity of NexGard against *S. calcitrans* was assessed 48, 72 and 96 hours after each challenge on Days 1, 7, 14, 21 and 28.

The efficacy was based on the survival rate of incubated live fed flies after 48, 72 and 96 hours. As the numbers of live fed flies incubated after the 30-min challenges were different for each dog and at each weekly incubation, the efficacy was calculated on the basis of proportions and not counts. The individual proportions at the 48, 72, and 96 hours time-points were calculated as the number of live fed flies/the number of live fed flies incubated after the 30-min challenges for each dog. The weekly efficacies at 48, 72, and 96 hours were calculated on the basis of the formula $100 \times [(C - T)/C]$, where C is the average proportion of live fed stable flies in the control group, and T is the average proportion of live fed stable flies in the treated group.

The groups were compared using a non-parametric Wilcoxon rank sum test on untransformed stable fly data. All statistical comparisons used a 5% significance level.

3. Results and discussion

No adverse reaction was observed in any of the NexGard®-treated dogs and no significant fly bite lesion was observed.

At the end of the five weekly 30-min challenges, the proportions of incubated live fed flies per group, relative to the total number of flies used for the challenges ranged from 40 to 77% without significant group difference, which demonstrated a reliable study model and no treatment effect after 30 minutes of exposure.

After the five weekly 48-h, 72-h and 96-h incubations, in the untreated control group, the average proportions of surviving flies relative to the number of flies incubated after the 30-min challenges were 74.6%, 61.4% and 49.1% respectively, which demonstrated an acceptable survival rate of flies in the insectarium.

Live fed *S. calcitrans* counts and efficacy results after incubations are presented in Table 2. The insecticidal efficacy raised proportionally to the duration of incubation. After 96 hours of incubation, the efficacy of afoxolaner against fed *S. calcitrans*, following challenges on Days 1, 7, 14, 21 and 28 ranged from 76.4 to 98.5% and was significant ($P \leq 0.05$) at all time-points.

Table 1
Animal and treatment details.

Animal ID	Age at inclusion (months)	Sex	Breed	Body weight at inclusion (kg)	Treatment	Afoxolaner dose (mg/kg)
Control/#1	45	Female	Beagle	14.3	Not applicable	Not applicable
Control/#2	62	Female	Beagle	14.1		
Control/#3	45	Female	Beagle	14.1		
Control/#4	41	Female	Beagle	14.5		
Control/#5	31	Male	Mongrel	18.6		
Control/#6	40	Male	Mongrel	16.1		
Control/#7	34	Female	Beagle	15.3		
NexGard/#1	92	Male	Mongrel	17.0	NexGard®	4.0
NexGard/#2	70	Male	Beagle	14.1		4.82
NexGard/#3	72	Female	Beagle	14.5		4.69
NexGard/#4	60	Female	Beagle	15.9		4.28
NexGard/#5	43	Male	Beagle	15.2		4.49
NexGard/#6	40	Female	Mongrel	14.6		4.67
NexGard/#7	37	Male	Beagle	16.2		4.20

Table 2
Insecticidal activity evaluations of Nexgard® against *S. calcitrans*.

S. calcitrans challenge[a]	Duration of incubation (h)[b]	Group mean rates (%) of live fed *S. calcitrans*[c]		Efficacy[f]	*P*-value[g]
		Control group[d]	Treated group[e]		
Day 1	48	78.7	32.8	58.1	0.1098
	72	75.9	28.8	61.6	0.0727
	96	71.3	16.1	76.4	0.0105
Day 7	48	83.9	13.7	81.8	0.1063
	72	58.0	2.9	93.5	0.0260
	96	48.3	0.8	98.2	0.0083
Day 14	48	67.2	13.2	81.9	0.0036
	72	57.6	2.9	94.9	0.0024
	96	51.5	0.8	98.5	0.0025
Day 21	48	64.2	27.1	60.1	0.1571
	72	52.6	8.9	82.7	0.0242
	96	19.0	2.1	89.9	0.0107
Day 28	48	79.3	30.4	57.0	0.0297
	72	62.8	7.5	86.2	0.0058
	96	55.6	3.1	94.6	0.0023

[a] Dogs were exposed to 50 ± 5 *S. calcitrans* for 30 min.
[b] Live fed *S. calcitrans* were collected after challenge and incubated for 48 h, 72 h and 96 h.
[c] Percentage of live fed *S. calcitrans* in relation to the number of live fed flies incubated after the 30-min challenge, individual values averaged per group.
[d] Control group: dogs were untreated.
[e] Treated group: dogs were treated once on Day 0 with NexGard® per label instructions.
[f] Percent efficacies calculated as 100 × [(C − T)/C], where C is the arithmetic mean of live fed *S. calcitrans* in the control group, and T is the arithmetic mean of live fed *S. calcitrans* in the treated group, proportional to the number of live fed flies incubated after 30 min of challenge in their respective groups.
[g] *P*-value: Wilcoxon rank sum test.

4. Conclusions

This study demonstrated insecticidal activity of NexGard® against *S. calcitrans* feeding on treated dogs. Although dogs are not the main host of *S. calcitrans*, treating dogs with NexGard® may decrease the number of flies staying in the vicinity of dogs, thus reducing the number of bites and dermatological consequences.

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Ethical approval

The study plan had been approved by the sponsor's and investigator's institutional animal care and use committee. Animals were handled similarly and with due regard for their wellbeing.

CRediT author statement

Eric Tielemans: Methodology, Writing - Reviewing and Editing. Nesrine Aouiche: Visualisation, Writing - original draft preparation. Adriaan Saunders: Visualisation, Investigation, Methodology. JF Besselaar: Data analysis, Methodology. Frédéric Beugnet: Supervision. All authors read and approved the final manuscript.

Disclaimer

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Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eric Tielemans, Nesrine Aouiche and Frederic Beugnet are current employees of Boehringer Ingelheim Animal Health, the owner of the investigated product (NexGard®) and the Sponsor of the study. Adriaan Saunders is current employee of ClinVet International. JF Besselaar is current employee of ClinData International. ClinVet International and ClinData International (Contract Research Organisations) were funded by Boehringer Ingelheim Animal Health for the conduct of the study.

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