curves at 0.1 µg/µl (10-fold the amount coated). *Alternaria alternata* extract was included in each Inhibition-ELISA assay as a non-inhibitory, negative control. The extract amount (µg) required to reach 50% of inhibition (i50) was calculated in each case in order to compare their inhibitory capacity. Inhibition-Immunoblottings were also performed. Briefly, proteins were immobilized in PVDF membranes and were incubated with pool previously co-incubated with three different concentrations of the same immobilized and the other two extracts. Concentrations ranged from the same strip-fixed amount to 100-fold less.

RESULTS

Inhibition-ELISA assays showed a high inhibitory capacity of the three extracts regardless the one coated. Maximum inhibition percentages of the three species were between 60 and 90%. When *D. farinae* was used in the solid phase, it yielded

the lowest i50 (0.049 µg) as inhibitor, which means the highest inhibitory capacity. When *A. siro* was coated it also showed the highest inhibitory capacity with the lowest i50 (0.411 µg). On the contrary, when *T. putrescentiae* was coated, *D. farinae* showed the lowest i50 (0.185 µg) whereas *T. putrescentiae* presented the highest i50 (0.344 µg). *A. alternata* had no inhibitory effect on any of the tested mite extracts. Inhibition-Immunoblotting showed a signal reduction at high concentration of inhibitor with the three mites, but with slight differences depending on the immobilized extract.

CONCLUSIONS

Using this positive serum pool we have observed a high cross-reactivity among these three mite species, although with slight differences, that could explain the high observed positivity concordance. Further studies with additional sera would be necessary to confirm these results.

Distribution and persistency of dinotefuran and pyriproxyfen in the hairs of cats topically treated with Vectra Felis

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INTRODUCTION

The efficacy and performances of topical ectoparasiticides are linked to the ability of the active ingredients to distribute onto the animal body and to persist in hairs. After administration on the skin in one single location of the body, the actives must move and provide a rapid protection to the whole body surface. These ingredients are also expected to persist in the animals hairs until the next administration. This study was designed to assess the kinetics of distribution and persistency in the hairs of the active ingredients from a topical combination of dinotefuran- pyriproxyfen (Vectra®Felis, DP) on cats.

METHODS

This study was performed according the principles of Good Laboratory Practices. After at least 6 days of acclimatization, adult cats were allocated to 2 groups: a control group (n=2, 3.6±0.5 kg BW) and a DP treated group (n=6, 4.3±0.6 kg BW). Cats in the treated group were administered 0.9 mL of DP while cats in the control group were administered 0.9 mL of a placebo on day 0. Hair samples were collected from cats with electric clippers in 5 body areas: the neck (excluding administration site), the middle of the back, the right and left sides of the thorax and the lumbar area. Sampling was performed at 7 time-points: before treatment, 1, 3, 7, 14, 21 and

30 days post-treatment. Dinotefuran and pyriproxyfen concentrations were measured in each sample using a validated HPLC/MSMS method. Results were considered only if in the analytical sequence results of calibration curves and QCs were within the accepted criteria. Cats were under general health observation for the duration of the study. This protocol has been previously approved by an ethical committee.

RESULTS

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In the control group, concentrations were below the limit of quantification for dinotefuran (< 1250 ng/g) and pyriproxyfen (<400 ng/g) from days -1 to 30, with the exception of some transient insignificant detectable amounts that were attributed to contamination. In the DP treated group, dinotefuran and pyriproxyfen were rapidly detected in the different body locations in 24h post-administration. Maximal concentrations

were observed 24h after administration near the neck but after only 14 days in the middle of the back. High concentrations of both active ingredients remained in the hairs for 30 days post-administration. Such initial and residual concentrations are supportive of the performances of the product against insects. Additionally the treated cats tolerated the product very well.

Rapid killing of *Rhipicephalus sanguineus* ticks on dogs treated topically with a combination dinotefuran-permethrin-pyriproxyfen

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INTRODUCTION

Rhipicephalus sanguineus is a common tick parasite in dogs and is a known vector of *Ehrlichia canis* and *Babesia canis*. Prevention of infestation with these ticks is a very important part of reducing the chance of transmission of these diseases. This study was designed to assess the efficacy of a topical administration of a combination of dinotefuran-permethrin-pyriproxyfen (Vectra®3D, DPP) at 8 and 48 hours after infestation in comparison with a negative (untreated) control group.

METHODS

Sixteen purpose-bred laboratory dogs were used in this study, with eight untreated dogs acting as controls and eight dogs treated topically with DPP in accordance with the label. Dogs were infested with 50 unfed adult *R. sanguineus* ticks with a balanced sex ratio on days 7, 14, 21, and 28 after treatment. Ticks were counted *in situ* at 8 hours ($\pm$ 30 minutes) after each infestation by direct observation following parting of the hair coat and palpation. Ticks were then counted and removed at 48 hours ($\pm$ 2 hours) after each infestation. The ticks at each

count were categorized as live or dead and by attachment status (free, attached but not engorged, or attached and engorged). Efficacy was calculated based on both geometric and arithmetic means of the number of live ticks (regardless of attachment status) plus the number of dead engorged ticks.

RESULTS

The mean counts in the control dogs were adequate at all counting times. The DPP combination rapidly killed or prevented attachment of ticks with excellent efficacy at both 8 and 48 hours at all time points up to 28 days. This topical product can be used to rapidly expel or kill new infestations of ticks which are vectors of important canine diseases.

	8 hour counts on Day			
	7	14	21	28
Control	34.3	27.6	35.3	33.9
DPP	3.8	2.9	2.9	0.9
Geometric Mean Efficacy	92%	92%	95%	98%
Arithmetic Mean Efficacy	89%	90%	92%	97%

	48 hour counts on Day			
	9	16	23	30
Control	26.4	30.8	31.9	30.8
DPP	2.9	0.4	0.5	0.0
Geometric Mean Efficacy	99%	100%	98%	100%
Arithmetic Mean Efficacy	89%	99%	98%	100%

In vitro canine IgE cross-reactivity among grasses, weeds and trees pollens

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INTRODUCTION

A high proportion of dogs diagnosed with Canine Atopic Dermatitis (CAD) shows positive specific-IgE simultaneously against phylogenetically distant pollens. The aim of this study was to determine IgE cross-reactivity among grasses, weeds and trees applying *in vitro* techniques.

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