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COMPARATIVE ACARICIDAL EFFICACY OF THE TOPICALLY APPLIED COMBINATIONS FIPRONIL/(S)-METHOPRENE, PERMETHRIN/IMIDACLOPRID AND METAFLUMIZONE/AMITRAZ AGAINST *DERMACENTOR RETICULATUS*, THE EUROPEAN DOG TICK (ORNATE DOG TICK, FABRICIUS, 1794) IN DOGS[1]

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Summary:

The acaricidal efficacy against *Dermacentor reticulatus* in dogs of the commercial topical combinations fipronil/(S)-methoprene (FRONTLINE Combo® spot-on dog), imidacloprid/permethrin (Advantix®) and metaflumizone/amitraz (ProMeris Duo®) was evaluated and compared. Three treatment groups and one untreated control group of six adult Beagle dogs each were randomly formed. Each treatment was administered topically once on Day-0, according to the recommended label dose and instructions for use. All dogs were infested weekly with approximately 50 adult unfed *D. reticulatus* over a period of seven weeks. Ticks were removed and counted approximately 48 hours after each infestation. The percent reduction in numbers of ticks for fipronil/(S)-methoprene was ⩾ 97 % compared to untreated controls for all seven weekly infestations. The percent reductions for imidacloprid/permethrin and metaflumizone/amitraz were satisfactory initially but fell and stayed below 90 % after three weeks. From the third week onwards, fipronil/(S)-methoprene treated dogs had significantly fewer ticks than imidacloprid/permethrin or metaflumizone/amitraz treated dogs ($p < 0.05$).

KEY WORDS: tick, *Dermacentor reticulatus*, dog, efficacy, fipronil/(S)-methoprene, imidacloprid/permethrin, metaflumizone/amitraz, experimental infestation.

Résumé : COMPARAISON DE L'EFFICACITÉ ACARICIDE CHEZ LE CHIEN DES FORMULATIONS TOPIQUES FIPRONIL/(S)-METHOPRENE, (FRONTLINE COMBO® SPOT-ON DOG), IMIDACLOPRID/PERMETHRIN (ADVANTIX®) ET METAFLUMIZONE/AMITRAZ (PROMERIS DUO®) CONTRE *DERMACENTOR RETICULATUS*, LA TIQUE EUROPÉENNE DU CHIEN (ORNATE DOG TICK, FABRICIUS, 1794)

Les efficacités acaricide contre Dermacentor reticulatus *chez le chien de formulations commerciales à application topique – fipronil/(S)-methoprene, imidacloprid/permethrin et metaflumizone/amitraz – ont été évaluées et comparées. Trois groupes traités avec chacune des formulations commerciales et un groupe contrôle non traité de six chiens adultes Beagle chacuns ont été constitués. Chaque traitement a été administré par voie topique une fois au Jour 0, en respectant les doses et les instructions d'utilisation du fabriquant. Tous les chiens ont été infestés une fois par semaine pendant sept semaines avec approximativement 50* D. reticulatus *adultes non nourris. Les tiques ont été retirées et comptées approximativement 48 heures après chaque infestation. Le pourcentage de réduction en nombre de tiques pour fipronil/(S)-methoprene en comparaison avec le groupe contrôle non-traité était de ⩾ 97 % pendant les sept semaines. Les pourcentages de réduction pour imidacloprid/permethrin et metaflumizone/amitraz étaient satisfaisants initialement, mais tombèrent sous 90 % après trois semaines. À partir de la troisième semaine et jusqu'à la fin de l'étude, les chiens traités avec fipronil/(S)-methoprene avaient un nombre significativement moindre de tiques que ceux traités avec imidacloprid/permethrin ou metaflumizone/amitraz ($p < 0.05$).*

MOTS CLÉS : *Tique,* Dermacentor reticulatus, *chien, efficacité, fipronil/(S)-methoprene, imidacloprid/permethrin, metaflumizone/amitraz, infestation expérimentale.*

INTRODUCTION

Dermacentor tick species are present worldwide (Wall & Shearer, 2001) and can be vectors of diseases for humans and animals. In Western Europe, *Dermacentor reticulatus* is a common tick species affecting dogs and is the primary vector of *Babesia canis* (Beugnet & Marié, 2009; Otranto & Dantas-Torres, 2010; Trotz-Williams & Trees, 2003). Effective control of tick infestation in dogs is based on several strategies, including avoidance of infested environments and regular application of acaricides (Otranto *et al.*, 2009). Effective environmental control measures are complex and have variable success rates. An infested environment is not easily identifiable and avoidance is not always possible with dogs. Regular applications of acaricide, or combination products (*e.g.,* insecticide/acaricide) are often used to control external parasite infestations in domestic animals. Spot-on or topical formulations of insecticide and/or acaricide drugs provide a convenient method for external parasite control in both dogs and cats.

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In this study, three veterinary-dispensed topical insecticide/acaricide combination products (Dryden *et al.,* 2008; Doyle *et al.,* 2005; Epe *et al.,* 2003; Hellman *et al.,* 2003; Rugg *et al.*, 2007) with labelled activity against *Dermacentor* were chosen for comparison: fipronil/(S)-methoprene (fipronil 10 % w/v + (S)-methoprene 9 % w/v; FRONTLINE Combo® spot-on dog [Europe] or FRONTLINE PLUS® [United States], Merial), imidacloprid/permethrin (imidacloprid 10 % w/v + permethrin 50 % w/v; Advantix® [Europe; Bayer AG] or K9 Advantix® [United States; Bayer Animal Health]), and metaflumizone/amitraz (metaflumizone 15 % w/v + amitraz 15 % w/v; ProMeris Duo® [Europe] or ProMeris® for dogs [United States], Fort Dodge Animal Health).
This study was designed to compare the efficacy of the fipronil/(S)-methoprene, imidacloprid/permethrin and metaflumizone/amitraz combinations against *D. reticulatus*.

MATERIALS AND METHODS

Experimental animals and husbandry

Twenty-four healthy adult beagle dogs (12 males and 12 females, weighing 8.3 to 15.2 kg and aged 14 to 52 months on Day-1) were studied. The dogs were housed individually in a controlled environment. Animal and allocation details are listed in Table I. Animals were managed similarly and with due regard for their welfare and in accordance with current local requirements. The husbandry conditions under which the animals were maintained were in compliance with the Statutory Instrument S.I. No. 566 of 2002, which incorporates EC directive, 86/609/EEC into Irish law.

Study design

This study was a blinded, randomized, single center, negative controlled, efficacy study performed at Charles River Laboratories Preclinical Services Ireland Ltd, Glenamoy; Co. Mayo; Ireland. A randomized block design based on pre-treatment tick count within sex was used. Six replicates of four dogs each were formed based on decreasing tick count. Within replicates, each dog was randomly allocated to one of the four treatment groups: Group 1: untreated control; Group 2: fipronil/(S)-methoprene (FRONTLINE Combo® spot-on dog); Group 3: imidacloprid/permethrin (Advantix®); Group 4: metaflumizone/amitraz (ProMeris Duo®).
Dogs were weighed on Day-1 for dose calculation purposes and treated on Day-0 according to the recommended label dose and instructions for use of each respective product. The treatment design is described in Table II.

For efficacy assessment, dogs were infested with 50 adult unfed *D. reticulatus* on Days 1, 7, 14, 21, 28, 35, and 42. Ticks were removed and counted 48 ± 2 hours after infestation, on Days 3, 9, 16, 23, 30, 37, and 44 (Table III). Ticks were categorised as 'live free', 'live attached un-engorged', 'live attached engorged', 'dead free', 'dead attached un-engorged', and 'dead attached engorged'. Ticks in the three 'live' categories, as well as in the 'dead, attached, engorged' category, were interpreted as treatment failures (EMEA/CVMP/005/2000, 2007; Marchiondo *et al.*, 2007), the counts were combined and the total was used in the efficacy analysis.

Treatment group	Tick count (Day-18)	Age (months) (Day-1)	Sex	Bodyweight (kg) (Day-1)
Untreated	39	31	Male	15.0
	35	18	Male	15.2
	24	18	Male	14.4
	37	17	Female	13.6
	33	18	Female	13.4
	29	18	Female	15.2
Fipronil (S)-methoprene	39	37	Male	13.9
	28	22	Male	11.8
	27	24	Male	12.7
	36	18	Female	14.5
	35	15	Female	11.0
	25	46	Female	12.3
Imidacloprid permethrin	36	52	Male	14.6
	33	23	Male	14.9
	25	24	Male	11.2
	36	16	Female	9.9
	35	44	Female	10.5
	31	15	Female	11.4
Metaflumizone amitraz	41	18	Male	9.8
	30	14	Male	14.6
	27	21	Male	13.9
	38	17	Female	8.3
	35	17	Female	10.4
	31	18	Female	10.1

Table I. – Animal and allocation details.

Treatment group	Weight of dog (kg)			Dose volume (mL)	
Untreated	NA			NA	
Fipronil (S)-methoprene	10	-	20	1.34	(1 pipette)
Imidacloprid permethrin	4	-	< 10	1.0	(1 pipette)
	10	-	< 25	2.5	(1 pipette)
Metaflumizone amitraz	5.1	-	10.0	1.34	(1 pipette)
	10.1	-	25.0	3.33	(1 pipette)

Table II. – Treatment design.
Each dog (except untreated controls) was treated once on Day-0 by the topical route and according to the instruction of use of the relevant Test item.

Treatment group	Tick category	Days of tick counts						
		Day-3	Day-9	Day-16	Day-23	Day-30	Day-37	Day-44
Untreated	Lf	0.1	0.0	0.0	0.0	0.1	0.3	0.1
	Lu	0.5	0.0	0.3	0.1	0.0	0.0	0.1
	Le	18.6	22.5	23.2	22.2	20.0	14.9	15.9
	Df	0.1	0.3	0.0	0.2	0.1	0.3	0.2
	Du	0.6	0.1	0.4	0.3	0.3	0.3	0.5
	De	0.3	0.3	0.6	0.5	0.7	0.5	1.0
Fipronil (S)-methoprene	Lf	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	Lu	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Le	0.0	0.0	0.0	0.0	0.0	0.1	0.3
	Df	0.3	0.1	0.3	0.1	0.4	0.7	0.4
	Du	0.4	0.1	0.7	0.0	0.1	0.1	0.3
	De	0.0	0.0	0.0	0.3	0.0	0.0	0.1
Imidacloprid permethrin	Lf	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Lu	0.1	0.0	0.0	0.0	0.0	0.0	0.1
	Le	1.3	1.6	0.6	3.1	4.7	3.6	7.8
	Df	0.1	0.5	0.3	0.1	0.1	0.7	0.7
	Du	0.0	0.7	0.1	0.3	0.4	0.1	0.7
	De	0.0	0.1	0.0	0.0	0.2	0.0	0.1
Metaflumizone amitraz	Lf	0.0	0.0	0.0	0.4	0.1	0.7	0.2
	Lu	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Le	0.2	0.0	0.1	3.9	6.4	7.5	15.2
	Df	0.1	0.8	0.0	0.4	0.0	0.2	0.3
	Du	0.1	0.0	0.0	0.1	0.4	0.4	0.6
	De	0.0	0.0	0.0	0.1	0.0	0.1	0.3

Tick status: Lf: LIVE Free ; Lu: LIVE Attached but Unengorged; Le: LIVE Attached but Engorged; Df: DEAD Free; Du: DEAD Attached but Unengorged; De: DEAD Attached but Engorged.

Table III. – Tick counts and categorization.
Geometric means by group, timepoint and category. Day-0 was the treatment day. Ticks were counted and categorised 48 ± 2 hours following infestation.

TICKS

Adult, unfed *D. reticulatus* of mixed sex ratio were used. Ticks were supplied from Charles River Laboratories Preclinical Service Ireland Ltd. The tick counts from the pre-treatment infestation showed that the strain was vigorous with > 34 % of ticks remaining on the untreated animals, which also demonstrated host suitability.

TICK INFESTATION AND COUNT PROCEDURES

Dogs were sedated, using xylazine and ketamine, for all infestations and counts. For all infestations, approximately 25 female and 25 male unfed *D. reticulatus* were placed on the left flank of each dog and allowed to crawl into the host's haircoat. Dogs were infested inside their respective pens. The sedation protocol was not reversed and the dogs remained motionless for one to three hours.

For tick removal and count procedures, dogs were placed on a table. Ticks were removed using individual tick extractors. All ticks were removed at each counting timepoint. The numbers of free (live or dead) ticks, and attached (live or dead) ticks were counted and recorded for each animal. The viability of the ticks found on dogs was evaluated by breathing on the tick and observing the presence or absence of reaction to this stimulation. Following removal, live attached ticks and dead attached ticks from each dog were collected in two separate containers. The evaluation of tick engorgement was based on the ingestion of blood by the ticks by squashing the tick on filter paper.

Gloves and protective clothing were changed between groups during the study and social interaction procedures to avoid cross-contamination. During the tick count procedures, oversuits and gloves were changed by the counting teams between groups, the linings of the tables where the sedated dogs were placed were changed between each group, and the dogs had their ticks removed with individual tick extractors, in order to decrease the potential for cross-contamination.

Day	Treatment group	Geometric mean[2]	Arithmetic mean[3]	Percent reduction[4]	Pairwise P-values[5,6]			
					Control	FsM	ImP	MeA
3	**Untreated**	20.2	21.3	--	--	<0.001	<0.001	<0.001
	Fipronil (S)-methoprene	0.0	0.0	100%	<0.001	--	0.007	0.363
	Imidacloprid permethrin	1.4	1.7	93%	<0.001	0.007	--	0.026
	Metaflumizone amitraz	0.2	0.3	99.0%	<0.001	0.363	0.026	--
9	**Untreated**	23.0	24.0	--	--	<0.001	0.004	<0.001
	Fipronil (S)-methoprene	0.0	0.0	100%	<0.001	--	0.087	1.000
	Imidacloprid permethrin	1.7	3.5	93%	0.004	0.087	--	0.087
	Metaflumizone amitraz	0.0	0.0	100%	<0.001	1.00	0.087	--
16	**Untreated**	24.2	24.3	--	--	<0.001	<0.001	<0.001
	Fipronil (S)-methoprene	0.0	0.0	100%	<0.001	--	0.084	0.363
	Imidacloprid permethrin	0.6	0.8	97%	<0.001	0.084	--	0.187
	Metaflumizone amitraz	0.1	0.2	99%	<0.001	0.363	0.187	--
23	**Untreated**	23.0	25.0	--	--	<0.001	0.001	0.001
	Fipronil (S)-methoprene	0.3	0.5	98%	<0.001	--	0.014	0.001
	Imidacloprid permethrin	3.1	4.2	87%	0.001	0.014	--	0.382
	Metaflumizone amitraz	4.8	5.7	79%	0.001	0.001	0.382	--
30	**Untreated**	21.4	21.8	--	--	<0.001	<0.001	0.056
	Fipronil (S)-methoprene	0.0	0.0	100%	<0.001	--	<0.001	0.006
	Imidacloprid permethrin	5.1	5.3	76%	<0.001	<0.001	--	0.669
	Metaflumizone amitraz	6.5	9.3	70%	0.056	0.006	0.669	--
37	**Untreated**	16.1	17.2	--	--	<0.001	0.004	0.208
	Fipronil (S)-methoprene	0.1	0.2	99%	<0.001	--	0.003	<0.001
	Imidacloprid permethrin	3.6	4.8	78%	0.004	0.003	--	0.044
	Metaflumizone amitraz	10.3	12.2	36%	0.208	<0.001	0.044	--
44	**Untreated**	17.3	18.5	--	--	<0.001	0.017	0.801
	Fipronil (S)-methoprene	0.6	1.0	97%	<0.001	--	0.001	<0.001
	Imidacloprid permethrin	8.4	9.0	52%	0.017	0.001	--	0.071
	Metaflumizone amitraz	16.0	18.2	7%	0.801	<0.001	0.071	--

1: For each dog at each sampling time, the numbers of counted ticks that fell into the categories, live and free, live and attached and unengorged, live and attached and engorged, or dead and attached and engorged, were summed together. These counts were then used for analysis. Each treatment group consisted of six dogs. 2: For each treatment group at each sampling time, the log-transformed data, $\log_e$[tick count + 1], were averaged and the geometric mean was computed as exp[x-bar] – 1, where x-bar was the mean of the transformed values. Note: not in statistical methods. 3: For each treatment group at each sampling time, the arithmetic mean of the tick counts was computed. 4: Percent reduction = 100 × [1 – T/C], where T and C are the geometric means of the treated and control groups, respectively. 5: Control = untreated, FsM = fipronil (S)-methoprene, ImP = imidacloprid permethrin, and MeA= metaflumizone amitraz. 6: Pairwise comparisons of the treatment groups within each sampling time. The log-transformed data was analyzed using Welch's two-sample t-test. This t-test adjusts the degrees of freedom based on the heterogeneity of the two samples variance.

Table IV – Results and Statistical Analysis
Analysis of Tick Counts over the Study Days[1]

STATISTICAL ANALYSIS

Ticks in the three 'live' categories, as well as in the 'dead, attached, engorged' category, were interpreted as treatment failures. Their counts were combined and the total was used in the subsequent analysis. The geometric means for each test group on each counting day were computed by averaging the log-transformed tick counts, $\log_e$[count + 1], exponentiating the result, and then subtracting 1. The percent reduction of the treatment groups with respect to the untreated control was calculated on each counting day using the formula 100 × [1-T/C], where T and C are the geometric tick count means of the treated and control groups, respectively.

Two hypotheses were tested: Hypothesis 1 was that Treatment Groups 2-4 had different expected tick counts from one another; Hypothesis 2 was that Treatment Groups 2-4 had lower expected tick counts than the Untreated Control Group 1. To test these hypotheses, Treatment Groups 2-4 were compared on a pairwise basis within each counting day, both amongst themselves as well as with Untreated Control. It was anticipated that there could be a high degree of heterogeneity of variances (because a treatment with a geometric tick count mean of 0 would have a sample variance of 0). Thus, the data were analyzed as if they had come from a completely randomized design. The MIXED procedure in SAS® Version 9.1.3 [SAS Institute, Inc.] was used on the log-transformed tick counts ($\log_e$[tick count + 1]). The model fitted included the Treatment as the fixed effect and as no random effect, and the "group=treatment" option was added to a repeated statement. This analysis provided the Welch's t-test for the pairwise comparisons (This statistic is a two-sample t-test with estimated degrees of freedom that were a function of the heterogeneity of the variances). All comparisons used the (two-sided) $\alpha = 0.05$ significance level.

RESULTS

The tick population was demonstrated vigorous as shown by tick attachment of > 34 % in the control animals throughout the study. The percent reduction for fipronil/(S)-methoprene did not fall below 97 % during the 44 days of the study, while the percent reductions for imidacloprid/permethrin and metaflumizone/amitraz both fell below 90 % on Day-23 and stayed below 90 % for the remainder of the study. The p-values from the pairwise comparisons amongst Treatment Groups 2-4, as well as with the untreated control Group 1, are listed in Table IV. From Day-23 onward, fipronil/(S)-methoprene had significantly fewer ticks than either imidacloprid/permethrin or metaflumizone/amitraz (*i.e.*, $p < 0.05$ for all comparisons). As

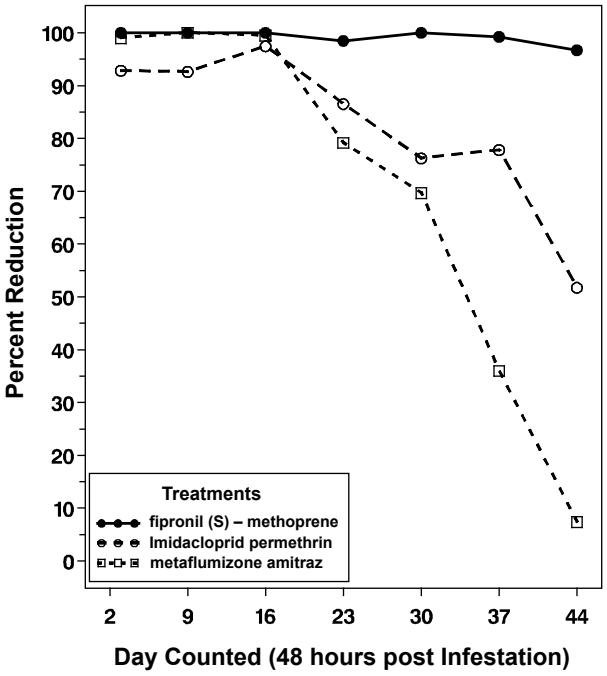

Fig. 1. – Percentage reduction results.

for comparisons with the untreated control, fipronil/(S)-methoprene and imidacloprid/permethrin had significantly fewer ticks than untreated control throughout the study ($p \leq 0.017$ for all Days), while metaflumizone/amitraz had significantly fewer ticks than untreated control on Day-23 or before.

DISCUSSION

Numerous *Dermacentor* species are present in Europe and Asia, including *D. reticulatus*, *D. marginatus, D. silvarum*, and *D. nuttalli* (Wall & Shearer, 2001). All these species have been described as vectors of disease of human and animal significance, like *Babesia canis, Ehrlichia chaffeensis* (granulocytic erhlichiosis), *Rickettsia rickettsii* (Rocky Mountain spotted fever), *Rickettsia slovaca* (TIBOLA), *Anaplasma marginale* (anaplasmosis), and Coltivirus (Colorado tick fever) (Beugnet & Marié, 2009; Otranto & Dantas-Torres, 2010; Trotz-Williams & Trees, 2003; Otranto *et al.*, 2009; Taboda & Lobetti, 2006). These diseases are rarely immediately transmitted, and there is commonly a lapse of time in transmission of the organism after tick attachment (Taboda & Lobetti, 2006; Kidd & Breitschwerdt, 2003). Therefore, effective tick controls for dogs and cats have a significant importance for public health and veterinary medicine, namely by decreasing vector-borne disease transmission.

In Western Europe, *D. reticulatus* is a common tick affecting dogs and is the primary vector of *B. canis*

(Beugnet & Marié, 2009). The documented transmission time for *B. canis* is two to three days (Kidd & Breitschwerdt, 2003). In this study, the efficacy assessment criteria were appropriate in the consideration of reduction of the potential for *B. canis* transmission by *D. reticulatus*.

This study confirmed that the fipronil-(S)-methoprene combination compared to the imidacloprid/permethrin and metaflumizone/amitraz combinations is longer acting and has a significantly higher ($p < 0.05$) killing effect on *D. reticulatus*.

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