

RESULTS OF A EUROPEAN MULTICENTRIC FIELD EFFICACY STUDY OF FIPRONIL-(S) METHOPRENE COMBINATION¹ ON FLEA INFESTATION OF DOGS AND CATS DURING 2009 SUMMER

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Summary:

Despite the increasing number of ectoparasiticides for pets and their use, flea infestations of cats and dogs are still widespread in Europe. It is therefore important to assess the maintenance of efficacy of the ectoparasiticides for cats and dogs. The present studies aimed to evaluate the efficacy of monthly treatments using a fipronil/(S)-methoprene combination spot-on (Frontline Combo®) on dogs and cats from private veterinary clinics located in seven European countries. The survey was conducted for three months during the flea season 2009. A total of 233 dogs and 180 cats were included. Each animal was treated at Days 0 (Day 0), 30 (D30) and 60 (D60) at the vet clinics. For each animal, at least three flea counts were performed at D0, D30 and/or D60 and/or day 90 (Day 90) in order to evaluate the prevalence of flea infestation and the efficacy of control. At the beginning of the study the prevalence of infested animals was 41.63 % (97/233) in dogs and 47.22 % (85/180) in cats. At D90, the number of dogs remaining infested fell to 8/211 therefore 91.75 % became flea-free. The number of infested cats fell from 85 to 9/173 at D90 therefore 89.41 % were cured. All animals still infested at Day 90 were living under epidemiological conditions that favour heavy flea burdens. These results are similar or better to previous studies, indicating the continuous high level of efficacy for fipronil 10 years after launch.

KEY WORDS: fleas, fipronil, field trial, efficacy.

Résumé : RÉSULTATS D'UNE ENQUÊTE MULTICENTRIQUE CONDUITE EN EUROPE DURANT L'ÉTÉ 2009 SUR L'EFFICACITÉ DE LA COMBINAISON FIPRONIL-(S) MÉTHOPRÈNE CONTRE L'INFESTATION DES CHIENS ET DES CHATS PAR LES PUCES

Malgré l'augmentation du nombre de produits vétérinaires anti-puces et de leur utilisation, l'infestation des chats et des chiens par les puces reste courante dans toute l'Europe. Il est donc important de vérifier la persistance de l'efficacité des antiparasitaires externes. La présente étude a pour objectif d'évaluer l'efficacité d'applications mensuelles de la combinaison fipronil/(S)-méthoprène en spot-on (Frontline Combo®) sur des chiens et des chats traités dans des clientèles vétérinaires privées de sept pays européens. Cette étude a été conduite durant trois mois pendant l'été 2009 (saison à puces). 233 chiens et 180 chats ont été inclus. Chaque animal était traité aux jours 0, 30 et 60 dans les cliniques vétérinaires. Pour chaque animal, au moins trois comptages de puces étaient réalisés à J0, J30, J60 et/ou J90 pour évaluer la persistance de l'infestation et l'efficacité du contrôle. Au début de l'étude, la prévalence d'infestation des animaux étaient de 41,63 % (97/233) chez les chiens et de 47,22 % (85/180) pour les chats. À J90, le nombre de chiens infestés chutait à 8 sur 211, par conséquent 91,75 % étaient devenus non infestés. Le nombre de chats infestés chutait de 85 à 9 sur 173 à J90 soit un taux de traitement de 89,41 %. Tous les animaux restant infestés à J90 vivaient dans des conditions épidémiologiques propices à des infestations importantes et régulières. Ces résultats sont similaires ou meilleurs que ceux d'enquêtes précédentes, ce qui démontre le haut niveau d'efficacité du fipronil 10 ans après sa mise sur le marché.

MOTS CLÉS : puces, fipronil, essai terrain, efficacité.

INTRODUCTION

Throughout the world, fleas, particularly those of *Ctenocephalides* genus, are the most common ectoparasites of dogs and cats. Flea infestation can cause considerable irritation to animals and humans and can lead to severe disorders such as anaemia and

dermatological problems including flea allergic dermatitis (FAD) (Dryden & Rust, 1994). It is estimated that flea related diseases are responsible for almost 50 % of dermatological cases reported to veterinarians. Many flea species are of veterinary and public health importance as they can be reservoirs and potential vectors for variety of pathogens including zoonotic agents. The cat flea, *Ctenocephalides felis* is a known vector for *Bartonella henselae*, *Bartonella clarridgeiae*, and *Rickettsia felis*, which in humans can cause cat scratch disease, endocarditis and cat flea typhus, respectively (Beugnet & Marie, 2009). The fleas of dogs and cats are also known as intermediate hosts of *Dipylidium caninum*.

For the last few decades flea species infesting pets have also become household pests with increasing

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¹ Frontline Combo® spot on. Merial

urbanization. For these reasons and because of animal and human welfare, the control of fleas is of great importance worldwide. Despite the increasing number of flea control products and their use, flea infestation of cats and dogs remains widespread in Europe and on other continents (Otranto *et al.*, 2009, part one and part two). It seems that completely eliminating them from companion animals in most cases remains a challenge. However, the efficiency of the current control measures can be improved using detailed epidemiological studies aimed to gather information about the most important flea species, their seasonality, and those factors which can influence their occurrence on dogs and cats living in urban and countryside areas (Otranto & Wall, 2008). The fipronil insecticidal spray and spot on formulations were launched in European countries almost 10 years ago. Frontline Combo[®], the combination of fipronil and (S)-methoprene, was launched in 2004 (Young *et al.*, 2004). Frontline Combo[®] spot on is a topical solution of fipronil (10 % w/v) and (S) methoprene (9 % w/v for the dog and 12 % w/v for the cat) (Young *et al.*, 2004). These two molecules diffuse within 24 hours on the body surface, which has been studied through radiolabelling. They are highly lipophilic and are stored in the skin oils for a long time. The dermal absorption is very limited and the combination is acting on arthropods by contact. Fipronil is acting on arthropod nerve cells through the glutamate-receptor and chloride channel pathway. Depending the natural susceptibility, fipronil maintains its complete efficacy on ticks for four weeks on dogs and two weeks on cats and on fleas from four to eight weeks on dogs.

S-methoprene is an analogue of the insect juvenile hormone. It limits the risk of environmental contamination by penetrating through the egg shell and inhibiting the larvae evolution into pupae. It acts when the concentration of fipronil decreases and when some adult fleas are able to survive and to lay eggs. S-methoprene has also the interest of inhibiting the appearance of new flea generation in case of any resistance selection to the insecticide. Based on the fact that pet owners want to deliver a complete protection for both fleas and ticks to their dogs and fleas to their cats, we decided to treat on a monthly basis.

It is useful to continuously assess the maintenance of efficacy of these ectoparasiticides for cats and dogs, especially when they are used broadly.

Experimental studies using laboratory flea strains are easier to perform, but surveys on the activity of a product in the field where flea populations are heterogeneous and where the modes of life of the carnivores are very variable are much more informative.

This study was conducted in collaboration with private veterinary clinics located in several European countries. The objective was to run a survey during the flea season

and to follow the impact of monthly treatments on the number of flea-free dogs and cats. It ran from late June until early November 2009. Previous flea surveys have demonstrated that the flea prevalence is increasing from Spring to Autumn with a peak in Autumn.

MATERIALS AND METHODS

LOCATION

Twenty-one veterinary clinics participated in this trial. They were located in seven European countries: Spain, France, Italy, Austria, Germany, Czech Republic and Hungary (Table I).

ANIMALS SELECTION

Ten to 30 dogs and 10 to 30 cats in each clinic were randomly selected by the veterinarians or the nurses, with the final acceptance of the owner (agreement form to be signed). Only healthy animals were included and animals that received an ectoparasiticide treatment in the previous two months were excluded. The flea infestation was not an inclusion criterion. For each selected animal, information about sex, age, breed, weight, life's style and parasitological status was collected during the first consultation. The history of antiparasitic treatments and ectoparasite infestations was also collected. An explanation of the flea biology as well as the prevention measures towards flea infestation was offered to the pets owners. The whole protocol and treatment used in the present study were also clearly explained.

TREATMENT

Frontline Combo[®] pipettes for dogs and cats were used according to the label and within the registered claims in EU for its official indication of flea and tick prevention of infestation. The expiry dates were checked by the veterinarians before application. The product applications were done by a trained person (nurse or veterinarian) every 30 days for three months at the vet clinic. The size of the pipette to be applied was decided after having weighted the animals.

PARASITOLOGICAL STATUS EVALUATION

The parasitological status was checked by combing the entire body of the animal according to previously described methodology (Gregory *et al.*, 1995). Briefly, the whole body of the animal was combed during a minimum of 7 minutes. If no fleas were found after 7 minutes, combing was discontinued at that time. If fleas were found, combing was continued until no flea was found for a period of 1 minute. If flea faeces were

Name of veterinarian or veterinary clinic	Location	Number of animals included
Dr E. Bibard	Dardilly, France	30 dogs / 30 cats
Dr V. Villeneuve	St Genis Laval, France	38 dogs / 21 cats
Ujpesti Allatorvosi Rendelo	Budapest, Hungary	6 dogs / 10 cats
Gaia	Szolnok, Hungary	1 dog / 1 cat
Nisa	Liberec, Czech Republic	19 dogs
Amb. Vet. Invrea - Chiartano	Italy	10 dogs / 10 cats
Amb. Basini Frola	Italy	10 dogs / 9 cats
Clin. San Francesco	Italy	9 dogs / 6 cats
Amb. Vet. Il Poggetto	Italy	10 dogs / 10 cats
Amb. Vet Masotti	Italy	10 dogs / 10 cats
Amb. Vet Venturini	Italy	8 dogs / 3 cats
Dr Schwarzmann	Austria	16 dogs / 14 dogs
C.V. San Marcos	Spain	5 dogs / 5 cats
C.V. Miami Platja	Spain	8 dogs / 5 cats
C.V. Canina y Felina	Spain	4 dogs / 3 cats
C.V. Beade	Spain	6 dogs / 4 cats
C.V. Sineu	Spain	5 dogs / 5 cats
C.V. Nova Veterinaria	Spain	5 dogs / 5 cats
Dr Weber & Thissen	Germany	15 dogs / 8 cats
Dr Möhnle	Germany	12 dogs / 11 cats
Dr Matzke	Germany	6 dogs / 10 cats
Total	21 veterinary clinics	233 dogs / 180 cats

Table I. – List of the veterinary clinics and their location

found, the animal was considered infested. Genus identification of the collected flea was done according to morphological criteria. In order to avoid variability induced by the number of investigators involved in flea combing, animals were classified into two groups: infested or “flea-free”. The precise charge of infestation through the number of fleas per animal was not recorded by all veterinarians at all time-counts.

STUDY SCHEDULE

Treatment was performed at days 0, 30 and 60. Flea counts were performed at Day 0, Day 30, Day 60, and Day 90 except for some animals which did not come for the last consultation (Table II). No control group was included as each animal was considered as its own control.

Date	Action
Visit 1: Day 0	Flea comb Frontline Combo® Treatment
Visit 2: Day 30	Flea Comb Questionnaire Frontline Combo® treatment
Visit 3: Day 60	Flea Comb Questionnaire Frontline Combo® treatment
Visit 4: Day 90	Flea Comb Questionnaire

Table II. – Design of the three month study.

RESULTS

SAMPLING

A total of 233 dogs with at least 3 flea counts were included and 211 of these dogs completed the 4 flea counts (Day 0, Day 30, Day 60 and Day 90). A total of 180 cats with at least 3 flea counts were included and 176 of these cats completed all counts. We lost a few pets that did not come probably due to the fact that there was no free treatment during the last visit. The highest parasitological charges recorded were observed in Spain. Those animals were all living with other pets in the presence of wild cats. All fleas checked were belonging to *Ctenocephalides* genus.

PREVALENCE OF FLEA INFESTATION IN DOGS

The prevalence of dogs infested by fleas at Day 0 was 41.63 % (97/233) (Table III). It fell to 3.79 % (8/211) at Day 90. The difference in the numbers of flea-infested dogs between each consultation time were significant except between Day 60 and Day 30 (Khi^2 , p-value < 0.05, see Table III). When considering the whole pet population, including both infested and non-infested dogs, we calculate an overall protection rate of 96.21 %. All the 136 non-infested dogs at Day 0 remained non-infested at Day 90 therefore the treatment provided a preventive efficacy of 100 % during the period at risk.

Country	Day 0		Day 30		Day 60		Day 90	
France	20/68	29.41%	1/66	1.51%	0/66	0%	0/66	0%
Hungary	0/7	0%	0/7	0%	0/7	0%	0/7	0%
Czech Republic	1/19	5.26%	1/19	5.26%	2/19*	10.52%	-	
Italy	24/57	42.10%	7/57	12.22%	6/57	10.52%	0/56	0%
Spain	24/33	72.72%	16/33	48.48%	11/33	33.33%	6/33	18.18%
Germany	20/33	60.60%	4/33	12.12%	4/33	12.12%	2/33	6.06%
Austria	8/16	50%	0/16	0%	0/16	0%	0/16	0%
Total	97/233	41.63%	29/231	12.55%	23/231	9.95%	8/211	3.79%
p-value (Khi ²)			Day 30 <i>versus</i> Day 0: 1.91 10 ⁻¹²		Day 60 <i>versus</i> Day 30: 0.377		Day 90 <i>versus</i> Day 60: 1.24 10 ⁻⁴⁴	

* Those two infested dogs were not infested at Day 60, whereas the one infested at Day 0 and Day 30 became non-infested. These two "new" infested dogs were located in a rural environment.

p-value from Khi² including all time points = 3.53 10⁻²⁹

Table III. – Results of flea infested dogs per country during the survey.

Country	Day 0		Day 30		Day 60		Day 90	
France	13/51	25.49%	1/49	2.04%	1/49	2.04%	1/49	2.04%
Hungary	0/11	0%	0/11	0%	0/11	0%	0/11	0%
Italy	21/48	43.75%	8/48	16.66%	0/47	0%	0/47	0%
Spain	19/27	70.337%	11/26	42.30%	9/26	34.61%	6/26	23.07%
Germany	22/29	75.86%	9/29	31.03%	5/29	17.24%	2/27	7.40%
Austria	10/14	71.42%	3/14	21.42%	0/14	0%	0/13	0%
Total	85/ 180	47.22%	32/177	18.07%	15/176	8.52%	9/173	5.20%
p-value (Khi ²)			Day 30 <i>versus</i> Day 0: 4.50 10 ⁻⁹		Day 60 <i>versus</i> Day 30: 0.008		Day 90 <i>versus</i> Day 60: 0.22	

p-value from Khi² including all time points = 2.77 10⁻²⁶

Table IV. – Results of flea infested cats per country during the survey.

Among the 97 infested dogs at Day 0, 81 (91.75 %) became flea-free at the end of the study and protected during the treatment period. The eight dogs that remained infested were all located in Spain or Germany. No persistence was observed in any of the other countries after three applications. Those dogs were all living in multi-pet households including cats, and had regular outdoor activities.

PREVALENCE OF FLEA INFESTATION IN CATS

The prevalence of cats infested by fleas at Day 0 was 47.22 % (85/180) (Table IV). It fell to 5.20 % (9/173) at Day 90, which indicates an overall protection rate of 94.8 %. The differences in the numbers of flea-infested cats between each consultation time were significant except between Day 90 and Day 60 (Khi², p-value < 0.05, see Table IV). All the 95 non-infested cats at Day 0 remained non-infested at Day 90 therefore treatment provided a preventive efficacy of 100 % during the period at risk assuming that they were facing some flea challenges. Among the 85 infested cats at Day 0, 76 (89.41 %) became flea-free at the end of the study and protected during the treatment period. The nine cats

that remained infested were located in Spain, Germany and France. The efficacy was 100 % in all other countries after three applications. Those cats were all living in multi-pet households with regular outdoor activities. During the three months of the study, the overall rate of flea infestation declined constantly either in dogs and cats (Fig. 1), which is in accordance with the epidemiological concept of environmental decontamination.

DISCUSSION

Based on epidemiology, this trial was conducted during the period with estimated maximum risk of flea infestation (Farkas *et al.*, 2009; Rinaldi *et al.*, 2007). Nevertheless, compared to recent epidemiological surveys conducted in Europe, the observed flea prevalence at Day 0 in this study was relatively high, with an average of 41.63 % for dogs and 47.22 % for cats. Typical summer rates are usually comprised between 8 to 22 % for dogs and 21 to 33 % for cats (Beck *et al.*, 2006; Bond *et al.*, 2005; Farkas *et al.*, 2009; Gracia *et al.*, 2009). The survey period corresponded

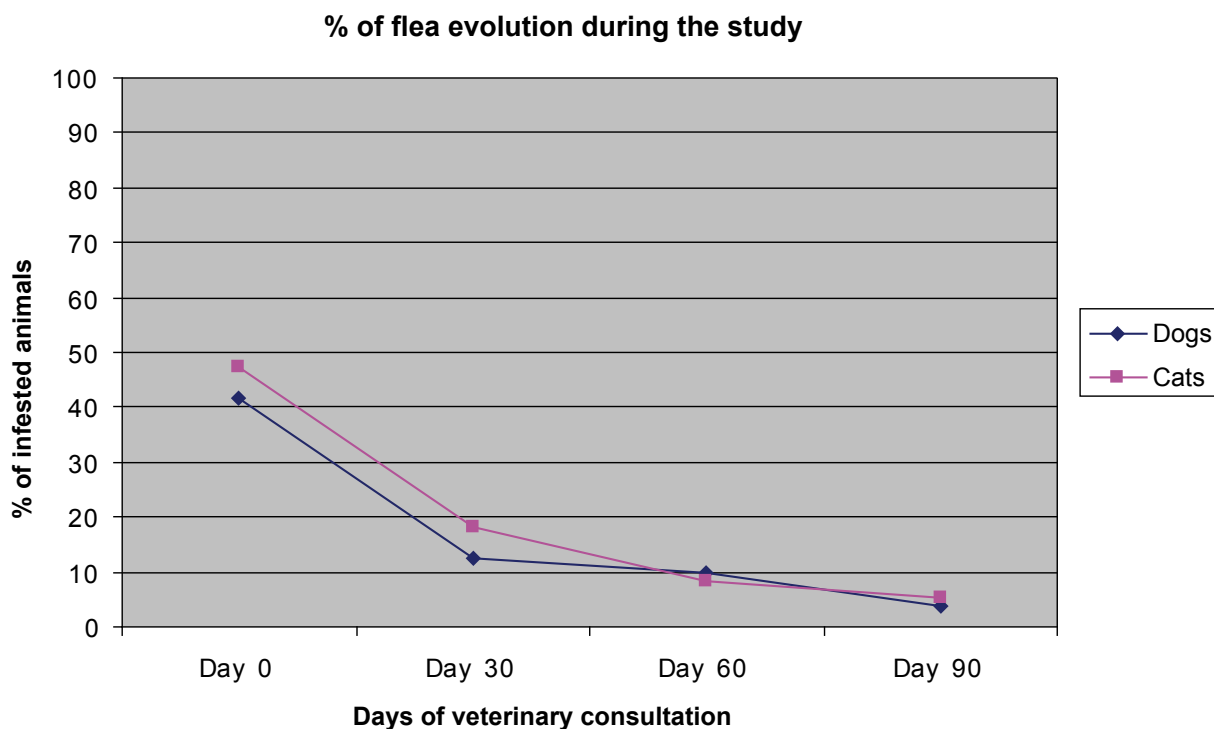


Fig 1. – Evolution of the rate of flea infestation during the three month survey.

to the highest slot for flea infestation, and the increase from Spring to Autumn has been documented by several authors and modeled in South of Italy (Gracia *et al.*, 2008; Rinaldi *et al.*, 2007).

One reason for the high infestation rates could be that the selected dogs and cats could not have been treated during the prior two months. This criterion may have selected for owners who do not treat regularly. The offer of three free treatments could also have led to the selection of owners who treat seldomly. Nevertheless, these flea infestation prevalences indicate that whatever the country, *Ctenocephalides* is a very common ectoparasite of both dogs and cats encountered by veterinarians.

The consistent decrease in the number of infested dogs or cats during the three months of the study demonstrates that some time is needed to decontaminate the environment. The elimination of flea pupae is progressively linked to their long life-span and their numbers which can be large (Dryden & Rust, 1994; Beugnet *et al.*, 2004). Other authors have estimated that two to four months of regular monthly treatments would be needed to achieve a flea-free environment (Jacobs *et al.*, 2001; Meo *et al.*, 1999; Krämer & Mencke, 2001). The combination of a long-acting insecticide with an insect growth regulator should accelerate this process. It may be longer in case of multi-pet households especially when animals have regular outdoor activities. In those cases, only a decrease in the flea numbers could be seen but not complete elimination (Dryden *et al.*,

2000). In our study, no environmental control was allowed, only the treatment of the pets with the topical combination of fipronil- (S) methoprene. All of the dogs or cats that remained infested were in a situation of high risk of fleas.

The “global” efficacy results of 96.21 % and 94.8 % for dogs and cats respectively, as well as the efficacy rates on infested animals of 91.75 % and 89.41 %, are similar to field trials conducted with fipronil or other insecticides in the past (Dryden *et al.*, 2000; Hellmann *et al.*, 2007; Hellmann *et al.*, 2003). In a trial conducted in Florida in 1999 including 88 households, after four fipronil applications and final observation at the end of the 5th month, 87.5 % of originally infested dogs and 77 % of originally infested cats were flea-free (Meo *et al.*, 1999). We obtained better results here with 91.75 % of the dogs becoming flea-free and 89.41 % of cats becoming flea-free. This study provides strong evidence that fipronil flea efficacy remains stable throughout Europe 10 years after launch.

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