**RESULTS OF A EUROPEAN MULTICENTRIC FIELD EFFICACY STUDY OF FIPRONIL-(S) METHOPRENE COMBINATION**\(^1\) **ON FLEA INFESTATION OF DOGS AND CATS DURING 2009 SUMMER**

**BEUGNET F.* & FRANC M.**

---

**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 98.41 % 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.

---

**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 dermatitits (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

---

* Merial, 29, avenue Tony Garnier, 69007 Lyon, France.
** Parasitology Department, National Veterinary School of Toulouse, 5, chemin des Capelles, 31076 Toulouse Cedex, France.
Correspondence: Frédéric Beugnet
E-mail: frederic.beugnet@merial.com
\(^1\) 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 radio-labelling. They are highly lipophilics 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 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
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 perform 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.

RESULTS

SAMPLING

A total of 233 dogs with at least 3 flea counts were included and 211 of these dogs completed all 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 (Khi², 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.
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 30 (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

<table>
<thead>
<tr>
<th>Country</th>
<th>Day 0</th>
<th>Day 30</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>20/68</td>
<td>1/66</td>
<td>0/66</td>
<td>0/66</td>
</tr>
<tr>
<td>Hungary</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>1/19</td>
<td>1/19</td>
<td>2/19*</td>
<td>2/19*</td>
</tr>
<tr>
<td>Italy</td>
<td>24/57</td>
<td>7/57</td>
<td>6/57</td>
<td>0/56</td>
</tr>
<tr>
<td>Spain</td>
<td>24/33</td>
<td>16/33</td>
<td>11/33</td>
<td>6/35</td>
</tr>
<tr>
<td>Germany</td>
<td>20/33</td>
<td>4/33</td>
<td>4/33</td>
<td>2/33</td>
</tr>
<tr>
<td>Austria</td>
<td>8/16</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>Total</td>
<td>97/233</td>
<td>29/231</td>
<td>23/231</td>
<td>8/211</td>
</tr>
</tbody>
</table>

p-value (Khi²) Day 30 versus Day 0: $1.91 \times 10^{-12}$

p-value (Khi²) Day 60 versus Day 30: $0.377$

p-value (Khi²) Day 90 versus Day 60: $1.24 \times 10^{-44}$

*p-value from Khi² including all time points = 3.53 $10^{-29}$

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Day 0</th>
<th>Day 30</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>13/51</td>
<td>1/49</td>
<td>1/49</td>
<td>1/49</td>
</tr>
<tr>
<td>Hungary</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
</tr>
<tr>
<td>Italy</td>
<td>21/48</td>
<td>8/48</td>
<td>9/26</td>
<td>9/26</td>
</tr>
<tr>
<td>Spain</td>
<td>19/27</td>
<td>11/26</td>
<td>5/29</td>
<td>2/27</td>
</tr>
<tr>
<td>Germany</td>
<td>22/29</td>
<td>9/29</td>
<td>0/14</td>
<td>0/13</td>
</tr>
<tr>
<td>Austria</td>
<td>10/14</td>
<td>3/14</td>
<td>32/177</td>
<td>15/176</td>
</tr>
<tr>
<td>Total</td>
<td>85/180</td>
<td>32/177</td>
<td>18.07%</td>
<td>5.20%</td>
</tr>
</tbody>
</table>

p-value (Khi²) Day 30 versus Day 0: $4.50 \times 10^{-9}$

p-value (Khi²) Day 60 versus Day 30: $0.008$

p-value (Khi²) Day 90 versus 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.
to the highest slot for flea infestation, and the increase from Spring to Autumn has been documented by several authors and modelized 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., 2005). 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.

**ACKNOWLEDGEMENTS**

The authors would like to thank all the veterinarians and their staff who participated to this trial seriously and enthusiastically. The authors thank also the pet owners who came back for the visits during the four months of the survey.

This research was funded by Merial S.A.S., Lyon, France.
REFERENCES

BECK W., BOCH K., MACKENSEN H., WIEGAND B. & PFIISTER K. Qualitative and quantitative observations of the flea population dynamics of dogs and cats in several areas in Germany. *Veterinary Parasitology*, 2006, 137, 130-136.


DRYDEN M.W., DENENBERG T.M. & BUNCH S. Control of fleas on naturally infested dogs and cats and in private residences with topical spot applications of fipronil or imidacloprid. *Veterinary Parasitology*, 2000, 93, 69-75.


MEO N., DRYDEN M., IRWIN J., RYAN W.G. & JEANNIN P. Monitoring of treated dogs and cats to assess flea control using monthly treatments of either fipronil spot-on or a combination of monthly lufenuron and pyrethroid spray treatments. 5th International Symposium on Ectoparasites of Pets (ISEP), April 1999, Fort Collins, Colorado, USA.


Reçu le 30 juin 2010
Accepté le 22 juillet 2010