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Evaluation of the efficacy and safety of a novel formulation of metaflumizone plus amitraz in dogs naturally infested with fleas and ticks in Europe

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Abstract

The efficacy and safety of a novel spot-on formulation of metaflumizone plus amitraz (ProMeris[®]/ProMeris Duo[®] for Dogs, Fort Dodge Animal Health, Overland Park, KS) was assessed in dogs naturally infested with ticks and/or fleas in a multiregional, clinical field study. Nineteen veterinary clinics in Germany and 11 clinics in France enrolled patients to the study. One hundred eighty one dogs with tick infestation and 170 dogs with flea infestation (plus three dogs harboring both ticks and fleas) qualified as primary patients and were randomly allocated to one of two treatments in a ratio of approximately 2:1 for metaflumizone plus amitraz (minimum dosage of 20 plus 20 mg/kg) or fipronil (at the recommended label rate). Clinical examinations and baseline parasite counts were performed on Day 0 prior to treatment. Tick and/or flea counts and safety evaluations were repeated at intervals of about 2 weeks for 8 weeks. Both products resulted in consistent reductions in tick numbers (>81%) throughout the study, with metaflumizone plus amitraz giving consistently higher reductions in tick numbers. The efficacy against tick count compared with Day 0 was 97.6%, 93.5%, 89% and 94% at Day 14, 28, 42 and 56, respectively, for metaflumizone plus amitraz. The corresponding efficacies for fipronil were 86.3%, 81.1%, 84.8% and 86.1%. Within groups, the tick reduction was highly significant ($P < 0.0001$) compared to baseline at all observation periods. Both treatments resulted in consistent (>89%) and highly significant ($P < 0.0001$) reductions in flea numbers relative to the baseline counts throughout the study, although fipronil resulted in numerically higher reductions on each count day. The efficacy against fleas compared to baseline was 91.8%, 88.7%, 91.5% and 92.0% at Day 14, 28, 42 and 56, respectively, for metaflumizone plus amitraz. The corresponding efficacies for fipronil were 98.2%, 96.3%, 95.9% and 96.7%. Metaflumizone plus amitraz was highly effective in controlling existing infestations of fleas and ticks on dogs and was effective against reinfestation for at least 56 days. Metaflumizone plus amitraz showed a good tolerance profile in dogs.

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1. Introduction

Tick and flea infestations are the major ectoparasites affecting dogs. The main flea species found on dogs is *Ctenocephalides felis felis* (Rust and Dryden, 1997; Beck et al., 2006) and the major tick species affecting

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dogs in Europe include *Ixodes ricinus* and *Rhipicephalus sanguineus* (Ogden et al., 2000; Földvári and Farkas, 2005). In Europe, ticks tend to occur on dogs from early spring to late autumn and are important vectors for several diseases affecting dogs (e.g. borreliosis, ehrlichiosis, tick encephalitis and babesiosis) and humans (e.g. tick encephalitis, borreliosis). Fleas tend to occur on dogs from spring to winter in Europe and are known to be vectors for other diseases. Therefore, treatment against fleas and ticks is important to prevent both canine and human disease.

Metaflumizone is a new insecticide in the semi-carbazone class of chemistry with potent activity against fleas (Takagi et al., 2007, this volume; Rugg and Hair, 2007, this volume) and no known cross-resistance to other chemistries (Salgado and Hayashi, 2007, this volume). Amitraz is a well-known formamidine acaricide (Hollingworth, 1976; Folz et al., 1986; Estrada-Pena and Ascher, 1999). A novel spot-on formulation containing metaflumizone plus amitraz (ProMeris[®]/ProMeris[®] Duo, Fort Dodge Animal Health, Overland Park, KS) applied as a single application to dogs to provide a minimum dose of 20 mg metaflumizone and 20 mg amitraz/kg provides at least 4 weeks control of fleas and ticks in laboratory studies (Rugg et al., 2007, this volume). The objective of this study was to evaluate the efficacy and persistency of the metaflumizone plus amitraz combination for the treatment of natural infestations of fleas and/or ticks in client-owned dogs presented as veterinary patients in Europe. This multi-center field clinical study was conducted according to Good Clinical Practice Guidelines (VICH, 2000) in veterinary clinics in Germany and France.

2. Materials and methods

2.1. Animals

Dogs brought to the participating clinics with tick and/or flea infestations were enrolled in the study. To be included in the study, dogs had to have ≥ 3 viable attached ticks and/or ≥ 5 viable fleas. Dogs were not eligible for the study, if they were < 10 weeks of age, < 2 kg bodyweight, puppies being nursed, lactating dogs or dogs for which mating was planned. Dogs with any history of apparent reactions to any spot-on parasitocidal treatment, dogs with pre-existing medical or surgical conditions other than tick and/or flea infestation or dogs which were bathed/shampooed within 48 h of treatment or were planned to be bathed/shampooed during the study were also excluded. The

animals remained with their owners under their usual housing conditions before, during and after the study.

2.2. Experimental design and methods

Nineteen veterinary clinics in four different areas (North, South, East and West) of Germany and 11 clinics in three areas (Central, West Coast and South) of France enrolled patients to the study. Enrolments were done from May to November 2004, a period when tick and flea infestations regularly occur in these geographical areas of Europe (Beck et al., 2006). Day 0 was defined as the day the animal presented to the clinic was initially identified, assessed and treated. On Day 0, prior to enrolment, the animal's details were recorded, a physical examination was performed to assess the general health of the animal, the suitability of the animal for the study was assessed, the relevant history recorded and the clinical observations including parasite counts on the whole body surface, using a standardized comb-count procedure, were done. Each animal was assigned a unique identification number and informed owner consent was obtained. Ongoing flea and tick challenge was monitored by observing other clients presenting their pets to the clinics during the time of the study.

Dogs infested with ≥ 3 viable attached ticks were selected as tick patients and allocated to treatment group according to a randomization list for tick patients. Dogs with ≥ 5 viable fleas were selected as flea patients and allocated to treatment according to a randomization list for flea patients. Animals were allocated to treatment with metaflumizone plus amitraz or fipronil in the ratio of 2:1. The same animal could be included as both a flea and tick patient if an adequate infestation of both parasites was present at the time of enrolment. Only animals from households with a maximum of four dogs/household or five animals/household (including cats) were considered for enrolment in the study. For tick efficacy, up to three qualifying dogs per household (primary animals) were enrolled. For the evaluation of efficacy in fleas, only one dog per household (primary animal) was enrolled. Other dogs (secondary animals) in the household were treated with the same product as the primary dog. Cats were treated with a registered flea control product. Primary patients were used for efficacy evaluation, while both primary and secondary patients were included in the safety evaluation.

To reduce bias, the study was blinded by using separate personnel to allocate and treat animals (Dispenser), and to conduct the parasite counts and clinical observations (Veterinarian) so that the latter remained blinded to treatment groups.

Parasite counts and clinical examinations were performed at study enrolment on Day 0 and when the clients returned the animals to the clinic on Days 14 ± 2 , 28 ± 2 , 42 ± 2 , and 56 ± 2 . To evaluate tick and flea infestations, dogs were subjected to a full body count. For ticks, the hair of the dog was pushed against its natural nap to expose the skin, ticks were removed using forceps, assessed for viability and placed in separate vials (dead or viable). Ticks were stored in the vials with alcohol and retained for later identification. Flea counts were conducted using a standard flea comb, with animals combed over the entire body for at least 5 min. During the process, fleas were removed from the dog and checked for viability (determined by observation of leg movement). Parasite counts were recorded on a standardized form. Safety of the test product was observed, documented, summarised and descriptively analysed for all dogs treated based on findings of abnormal health and the presence or absence of application site reactions during the study period, either observed by the examining Veterinarian or reported by the owner.

2.3. Treatment

Animals of both treatment groups were treated once on Day 0 according to their individual bodyweight. Following allocation, the Dispenser either treated the animal or supervised the treatment by the animal owner. Metaflumizone plus amitraz was administered as a unit dose, providing the recommended minimum dose of 20 mg metaflumizone plus 20 mg amitraz/kg, according to the following weight bands: ≤ 5.2 kg, 0.7 ml; 5.3–10.5 kg, 1.4 ml; 10.6–25.5 kg, 3.4 ml; 25.6–40.5 kg, 5.4 ml; 40.6–51.0 kg, 6.8 ml; dogs > 51.0 kg were treated with the appropriate combination of the smaller weight bands. Individual dog doses were supplied in glass vials and the appropriate dose was drawn in to a suitably sized disposable syringe. The entire dose was applied using the syringe to a single spot on the skin between the scapulae. The reference product (Frontline® Top Spot, Merial, Duluth, GA) was applied following label directions using the commercial applicator in a similar fashion to provide a minimum dose of at least 6.7 mg fipronil/kg.

2.4. Data analysis

Calculations and analyses were performed using SAS® statistical analysis software (SAS Institute Inc., Cary, NC, USA, release Version 8.2). A natural logarithmic transformation $\{\ln(\text{count} + 1)\}$

was applied to flea and tick counts prior to analysis. Geometric mean(gm) flea and tick counts were calculated from the least square means of the analysis of variance with treatment and assessment day as variables. These were used to estimate percentage reductions of flea and tick counts within each treatment relative to baseline (Day 0) using the following formula:

$$\% \text{Efficacy} = \frac{\text{gm Day 0} - \text{gm assessment day}}{\text{gm Day 0}} \times 100$$

Non-inferiority of ln-transformed mean tick counts for dogs treated with metaflumizone/amitraz compared with those for dogs treated with fipronil was assessed for each Day 14, 28, 42 and 56 using a non-inferiority margin (delta) of 0.49 and assuming that the control product, fipronil, had a mean count of 0.53 and a standard deviation of 1.17. Non-inferiority of ln-transformed flea counts were compared in the same manner using a delta of 0.46 and assuming the control product had a mean count of 0.72 and a standard deviation of 1.12. Within each treatment group, the ln-transformed counts of both ticks and fleas at each post-treatment time point was compared to Day 0 using a paired *T*-test.

3. Results

3.1. Animals

Nineteen practices in Germany and 11 in France enrolled patients to form the study population. Enrolments were completed from May to November 2004, a period when tick and flea infestations regularly occur in these geographical areas of Europe. In total, 442 individually identified dogs were treated; these comprised 181 tick patients, 170 primary flea patients and 91 supplementary dogs. The total number of dogs treated was considered the “safety” population; 293 were treated with metaflumizone plus amitraz and 149 with fipronil. Dogs were recruited from a range of geographical areas and varied living conditions, including single and multi-pet households and indoor and outdoor accommodation. Animal details are summarized in Table 1. The distributions of hair coat lengths were similar for dogs in both treatment groups (Table 2). Similarly, the proportions of dogs exposed to water, shampooing or environmental insecticide treatments, based on Day 0 information from clients, was consistent for dogs from both treatment groups (Table 3).

Table 1
Details of dogs enrolled in a multi-center field clinical study

Treatment	Sex no. (%)		Age in years, mean (range)	Bodyweight in kg, mean (range)	Breed no. (%)	
	Male	Female			Pure	Mixed
Tick patients						
Metaflumizone plus amitraz	65 (54.6)	55 (45.5)	4.5 (0.3–13)	27.5 (3.8–79.0)	72 (59.5)	49 (40.5)
Fipronil	22 (43.1)	29 (56.9)	5.2 (0.3–14)	24.4 (5.5–55.0)	41 (80.4)	10 (19.6)
Flea patients						
Metaflumizone plus amitraz	45 (39.5)	69 (60.5)	5.6 (0.2–13)	17.6 (2.1–49.9)	81 (71.0)	33 (29.0)
Fipronil	23 (40.4)	34 (59.7)	5.8 (0.2–14)	19.4 (2.6–58.9)	39 (68.4)	18 (31.6)

Table 2
Hair coat length of dogs enrolled in a multi-center field clinical study evaluating the efficacy and safety of a metaflumizone plus amitraz spot on formulation for the control of ticks and fleas

Treatment	Hair coat length, no. (%)		
	Long	Medium	Short
Tick patients			
Metaflumizone plus amitraz	19 (15.7)	54 (44.6)	48 (39.7)
Fipronil	9 (17.7)	20 (39.2)	22 (43.1)
Flea patients			
Metaflumizone plus amitraz	18 (15.8)	51 (44.7)	45 (39.5)
Fipronil	10 (17.5)	24 (42.1)	23 (40.4)

3.2. Efficacy against ticks

The major tick genera identified from dogs enrolled in the study were *Ixodes* spp. and *Rhipicephalus* spp. The mean tick count on Day 0 was 5.2 ticks per dog for both treatment groups (Table 4). Within groups, the reduction of tick counts following treatment was highly significant ($P < 0.0001$) compared to Day 0 at all time points for both metaflumizone plus amitraz and fipronil. Numerically, the reductions in overall tick numbers and each individual genus for dogs treated with metaflumizone plus amitraz were about 5–10% greater than those

for the control product at most assessments. Geometric mean tick counts, for dogs treated with metaflumizone plus amitraz were lower on Day 14 and Day 28 ($P < 0.05$) than those of dogs treated with the control product. On Days 42 and 56, both products resulted in similar tick counts ($P > 0.05$). Ongoing evaluation of other dogs presented to the clinics during the study indicated that there was a consistent tick challenge.

3.3. Efficacy against fleas

Fleas were not specifically identified during the study, but it was assumed that the predominant species was *C. felis felis*. The mean Day 0 flea counts were 14.3 and 15.4 fleas per dog for the metaflumizone plus amitraz and fipronil groups, respectively (Table 5). Ongoing evaluation of other dogs presented to the clinics during the study indicated that there was a consistent flea challenge. The efficacy of the metaflumizone plus amitraz treatment was relatively constant throughout the study. Within groups, the reduction of flea counts following treatment was highly significant ($P < 0.0001$) compared to Day 0 at all time points for both metaflumizone plus amitraz and fipronil. Although geometric mean flea counts for dogs treated with metaflumizone plus amitraz were higher ($P < 0.05$)

Table 3
Exposure to water by swimming or thorough wetting (at least monthly), shampooing or environmental parasite treatments for dogs enrolled in a multi-center field clinical study evaluating the efficacy and safety of a metaflumizone plus amitraz spot on formulation for the control of ticks and fleas

Treatment	Water exposure no. (%)		Shampooing no. (%)		Env. treatment no. (%)	
	Never	Monthly	Never	Monthly	Never	Monthly
Tick patients						
Metaflumizone plus amitraz	81 (66.9)	40 (33.1)	106 (87.6)	15 (12.4)	120 (99.2)	1 (0.8)
Fipronil	39 (76.5)	12 (23.5)	44 (86.3)	7 (13.7)	51 (100)	0 (0.0)
Flea patients						
Metaflumizone plus amitraz	92 (80.7)	22 (19.3)	93 (81.6)	21 (18.4)	112 (98.2)	2 (1.8)
Fipronil	46 (80.7)	11 (19.3)	45 (79.0)	10 (17.5)	55 (96.5)	2 (3.5)

Table 4
Geometric mean tick counts and percent efficacy in dogs following a single treatment with metaflumizone plus amitraz or fipronil

Study day	Metaflumizone plus amitraz			Fipronil		
	No.	Geo. mean tick count	%Efficacy	No.	Geo. mean tick count	%Efficacy
All ticks						
0	121	5.18	–	51	5.17	–
14	121	0.13*	97.6	51	0.71*	86.3
28	121	0.34*	93.5	51	0.98*	81.1
42	121	0.57*	89.0	50	0.79*	84.8
56	119	0.31*	94.0	50	0.72*	86.1
Ixodes spp.						
0	92	4.54	–	47	4.48	–
14	92	0.16*	96.5	47	0.70*	84.4
28	92	0.47*	89.7	47	1.00*	77.6
42	92	0.74*	83.7	47	0.80*	82.2
56	92	0.52*	88.4	47	0.83*	81.5
Rhipicephalus spp.						
0	26	9.64	–	6	4.08	–
14	26	0.26*	97.3	6	0.94*	76.9
28	26	0.15*	98.5	6	0.91*	77.8
42	26	0.26*	97.3	6	0.67*	83.5
56	26	0.03*	99.7	6	0.44*	89.2

* Tick counts significantly less than on Day 0 ($P < 0.0001$) for each tick category.

than those of dogs receiving control product at all post-treatment observations, efficacy was $>85\%$ for both groups throughout the study. General comments from the cooperating clinics also supported that there was an ongoing environmental flea challenge. Especially in the south of France, there were multiple reports by veterinarians that the turnover of flea products was very high and that registered products were not working as well as expected.

3.4. Health observations

Twelve animals out of the 293 treated with metaflumizone plus amitraz showed adverse reactions that might have been related to treatment. Six cases (2.04%) showed possible application site reactions. Of these, one dog showed a black, pigmented area at the spot-on region, one a sticky coat, one a clotted coat, two

dogs had bran like dandruff on dorsal midline, and another owner reported olfactory nuisance possibly due to an interaction with the dog's collar. Six cases (2.04%) included such signs such as vomitus, bradycardia, hypothermia, tiredness, languor, apathy, inappetence, diarrhoea, pruritus, and pustules on the abdomen. These were all generally mild, transient and all resolved. No suspected adverse reactions were reported for the 149 dogs treated with the fipronil spot-on.

4. Discussion

This field clinical study was conducted to confirm the efficacy and safety of a metaflumizone plus amitraz spot on formulation under normal use conditions in Europe. The study was conducted from April to November 2004 with a sustained parasite pressure. Both treatment groups were similar with regard to sex

Table 5
Geometric mean flea counts and percent efficacy in dogs following a single treatment with metaflumizone plus amitraz or fipronil

Study day	Metaflumizone plus amitraz			Fipronil		
	No.	Geo. mean tick count	%Efficacy	No.	Geo. mean tick count	%Efficacy
0	114	14.31	–	57	15.38	–
14	114	1.70*	88.1	57	0.47*	97.0
28	113	2.14*	85.1	56	0.87*	94.3
42	111	1.78*	87.6	56	1.06*	93.1
56	111	1.96*	86.3	56	1.09*	92.9

* Flea counts significantly less than on Day 0 ($P < 0.0001$).

ratio, age, bodyweight, hair length and water contact, and included a broad range of breeds. Following a single application, metaflumizone plus amitraz was effective in reducing tick and flea infestations for at least 56 days. Posttreatment efficacy against ticks ranged from 89.0% to 97.6% and was higher than that for the control product that had efficacies ranging from 81.1% to 86.3%. Both products had similar efficacy against the prevalent tick species (*Ixodes* spp. and *Rhipicephalus* spp.). Similarly, flea counts were effectively reduced or at least 56 days. Posttreatment efficacy for metaflumizone plus amitraz ranged from 85.1 to 88.1% and was still 86.1% at Day 56. Efficacy of the control product ranged from 97.0% on Day 14 to 92.9% on Day 56.

The differences seen between the efficacies of the two products for the different parasites probably relate to factors inherent to field clinical design and nature of the active ingredients. The combination of metaflumizone plus amitraz incorporates two different compounds with markedly different modes of action and speed of activity; metaflumizone is potent against fleas but has no significant activity versus ticks, while amitraz is a highly effective acaricide with no efficacy against fleas. Metaflumizone plus amitraz has been shown to control fleas for up to 8 weeks in laboratory studies with maximum efficacy (>95%) achieved by 24–48 h after infestation; at 8 h after infestation, efficacy ranged from 25% to 56% (unpublished data). These activity kinetics reflect the mode of action of metaflumizone which is a sodium channel blocker. Toxicity is manifest by a rapid cessation of feeding, followed by flaccid paralysis and eventual death (Salgado, 1990; Wing et al., 1998). Thus, affected fleas may be detected as live fleas for up to 24 h after reinfestation. A laboratory study also showed that fipronil provided >95% kill of fleas within 8 h after reinfestation, but that flea kill was similar to that for metaflumizone plus amitraz at 24 and 48 h after infestation (unpublished data). Thus, the flea efficacies seen in this study are consistent with the efficacies seen at 8–24 h after infestation in pen studies, indicating that some dogs may have been exposed to reinfestation pressure within 24 h prior to the clinic visit. As the levels of control for both products remained high and constant for the entire study, flea reinfestation pressure within the household environment should have been reduced. It has been shown that monthly spot-on insecticide treatments will provide excellent control of flea in simulated home environments (Hutchinson et al., 1998). Thus, it was likely that the live fleas detected at the posttreatment clinical exams were a recent reinfestation, possibly picked up within the previous 24 h outside the house from the environment when the

dog was walked. This supposition is supported by the results of a field clinical study with metaflumizone in cats where treatment resulted in consistent efficacy equivalent to the control product, fipronil, of around 90% (Hellmann et al., 2007, this volume). Cats are generally restricted to the home environment and thus less likely to encounter reinfestation from the external environment than dogs.

A similar pattern of interaction between the action of the different active ingredients and environmental exposure was possibly seen for tick efficacy. Here, the metaflumizone plus amitraz treatment had consistently higher efficacy than the fipronil treatment for the first 28 days. Both products have similar efficacy against ticks under laboratory challenge studies. The higher efficacy for the metaflumizone plus amitraz treatment in this clinical study might reflect the fact that amitraz is a relatively fast-acting acaricide, causes rapid detachment and has quicker kill than fipronil (Estrada-Pena and Ascher, 1999).

The patterns of efficacy seen in this study suggests that caution should be exercised in a clinic-based field study design, especially when evaluating compounds that may have a relatively slow rate of kill of fleas. If the dogs could have been maintained in the clinic for 24–48 h, this would have insured that newly infested fleas were exposed to the slower acting metaflumizone. The “in home” field clinical study design (Dryden et al., 1999, 2000) where the investigator evaluates the environment and flea burden in the actual household might provide a more accurate evaluation of field performance.

5. Conclusions

The results of this study showed that a metaflumizone plus amitraz spot-on administered at a minimum dose rate of 20 mg/kg for each active ingredient was highly effective (>97.6%) in controlling existing infestations of ticks and was highly effective (89–94%) in preventing tick reinfestation in dogs for 56 days following treatment. Based on the reduction in flea counts, metaflumizone plus amitraz efficacy was >85% and consistent throughout the study indicating effective control of fleas under field trial conditions. Metaflumizone plus amitraz showed a good tolerance profile in dogs.

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