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Confirmation of the efficacy of a novel formulation of metaflumizone plus amitraz for the treatment and control of fleas and ticks on dogs

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Abstract

A novel spot-on formulation containing metaflumizone plus amitraz (ProMeris®/ProMeris Duo® for Dogs, Fort Dodge Animal Health, Overland Park, KS) was evaluated in four laboratory studies to confirm efficacy against fleas and ticks on dogs for 1 month. Three different strains of cat flea (*Ctenocephalides felis felis*) and four tick species were used. *Rhipicephalus sanguineus* and *Dermacentor variabilis* were evaluated concurrently in two studies and *Ixodes scapularis* and *Amblyomma americanum* in one study each. In all studies, dogs were randomly allocated to treatment groups and compared with nontreated dogs. One study also included a placebo treatment and a commercial product containing fipronil plus S-methoprene. All treatments were applied to the skin at a single spot between the scapulae on Day 0. Dogs were infested with fleas and/or ticks prior to treatment and then reinfested at weekly intervals for 6 weeks after treatment and evaluated for efficacy at 1 or 2 days after treatment and each reinfestation. These studies confirmed that treatment with ProMeris for Dogs at the proposed commercial dose rate rapidly controlled existing infestations of fleas and ticks on dogs. Treatment provided control of reinfesting fleas for up to 6 weeks and at least 4 weeks control of ticks. Efficacy was confirmed in a variety of dog breeds against three different flea strains and four common species of ticks found on dogs in the United States.

Keywords: ProMeris®; ProMeris Duo®; *Ctenocephalides felis felis*; *Rhipicephalus sanguineus*; *Dermacentor variabilis*; *Ixodes scapularis*; *Amblyomma americanum*; Metaflumizone; Amitraz; Flea; Tick; Dog

1. Introduction

The cat flea *Ctenocephalides felis felis* is endemic worldwide and is considered the most important ectoparasite of dogs and cats (Rust and Dryden, 1997). Fleas are recognized as a major cause of allergic skin disease in dogs and are capable, when present in sufficient numbers, of causing anemia (Krämer and Menke, 2001). They are intermediate hosts for the cestode *Dipylidium caninum*, and can transmit a number of pathogens including *Bartonella henselae*. Infestations of ticks can have widely varying effects on dogs; ticks may be an occasional nuisance or a continuous infestation, and they may cause virtually no adverse effects on health or cause life-threatening disease. The genera of ticks commonly infesting dogs in the US are *Amblyomma*, *Dermacentor*, *Ixodes* and *Rhipicephalus* (Dryden and Payne, 2004). Some tick species excrete

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toxic substances within their saliva and tick-borne diseases may be passed to their next host in saliva (Needham and Teel, 1991). A large infestation of ticks may cause anemia and ticks are responsible for the transmission of a number of diseases, some are dog-specific, some are zoonotic and some cause serious, even life-threatening, diseases (Dryden and Payne, 2004).

Control of fleas and ticks is primarily based on chemical means and recently, convenient on animal treatments have become the standard accepted method (Dryden and Payne, 2004; Rust, 2005). Despite the variety of available products and different application methods, both fleas and ticks remain an ongoing problem for many pet owners. In addition, the baseline susceptibility or tolerance of different flea and tick populations and the tick species to individual insecticides and acaricides can vary widely (Bossard et al., 1998; Ross et al., 1998; Rust, 2005). The US EPA recommends that new insecticides be evaluated against at least three geographically distinct cat flea populations (US EPA Guideline, OPPTS 810.3300).

Metaflumizone is a novel insecticide in the semicarbazone class of chemistry with potent activity against fleas *in vitro* and *in vivo* via topical application to dogs and cats (Takagi et al., this volume; Rugg and Hair, this volume) and no known cross-resistance to other chemistries (Salgado and Hayashi, this volume). This compound was combined with the formamidine acaricide amitraz in a novel spot-on formulation (ProMeris®/ProMeris Duo® for Dogs, Fort Dodge Animal Health, Overland Park, KS) to develop a product for flea and tick control on dogs (Sabnis and Zupan, this volume). The appropriate dose rate of a formulation of metaflumizone and amitraz applied as a single spot application to dogs to provide at least a month of control of fleas and ticks study was determined in a previous study (Rugg and Hair, this volume). Here we report studies conducted to confirm the efficacy of this formulation applied as unit doses to dogs to control different flea strains and the major tick species parasitizing dogs in the US.

2. Materials and methods

Four studies were conducted examining the efficacy of ProMeris for Dogs applied at the proposed commercial dose rates against a number of cat flea strains (three studies) and/or different species of ticks at three different sites in the US. Two studies (A and B) were conducted at Nu-Era Farms Inc. (NEF), OK, another (C) at AgResearch Consultants Inc. (ARC), AR, and the fourth (D) at STILLMEADOW Inc. (STM), TX. All studies were conducted according to Good Laboratory Practices as outlined in US EPA 40CFR160, and followed the basic methodology of US EPA Guideline, OPPTS 810.3300.

2.1. Animals

2.1.1. Studies A and B

Eight male and eight female adult Beagle dogs from the NEF colony were used in each study. Each dog was individually identified by numbered or lettered ear tattoos. The dogs had not been treated with an ectoparasiticide for at least 30 days and were in good health when enrolled in the study and at treatment. The animals weighed from 7.3 to 16.1 kg on Day −2. These animals were selected from a group of nine male and nine female dogs (A) or 10 male and 10 female dogs (B) based on pretreatment flea and/or tick counts.

Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Each run was approximately 3 m × 1 m with wire mesh walls on epoxy-coated concrete flooring and contained a raised mesh rest. Dog runs were grouped by treatment. Each individual run was labeled with the dog identification number only and was not identified by treatment. Dogs from different treatment groups were physically separated by space equivalent to at least one empty run.

Dogs were fed an appropriate maintenance ration of a commercial dry canine feed (27% Hi-Protein Complete Ration, A+M Feeds, Stillwater, OK 74074) for the duration of the study. Water was available *ad libitum*.

2.1.2. Study C

Sixteen male and 16 female adult mixed breed dogs from the ARC colony were used in the study. Each dog was individually identified by numbered tags attached to a neck chain. The dogs had not been treated with an ectoparasiticide for at least 14 days and were in good health when enrolled in the study. The animals weighed from 5.4 to 30.6 kg on Day −2. These animals were selected from a group of 19 male and 21 female dogs based on pretreatment flea and tick counts, skin condition and health. All dogs included in the study retained >30 fleas and >5 ticks at the pretreatment evaluation. Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Each run was approximately 1.2 m × 1.2 m (small dogs) or 2.4 m × 1.2 m (large dogs) with wire mesh walls on concrete flooring. Dog runs were grouped by treatment. Each

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individual run was labeled with the dog identification number only and was not identified by treatment. Dogs from different treatment groups were physically separated by space equivalent to at least one empty run.

Dogs were fed an appropriate maintenance ration of a commercial dry canine feed (VF Complete®, Arkat Inc., Dumas, AR). Water was available *ad libitum*.

2.1.3. Study D

Eight male and eight female adult mixed breed dogs from the STM colony were used in the study. Each dog was individually identified by a unique ear tattoo. The dogs had not been treated with an ectoparasiticide for at least 30 days and were in good health when enrolled in the study. The animals weighed from 8.6 to 14.0 kg on Day −2. These animals were selected from a group of 9 male and 10 female dogs based on pretreatment flea counts. All dogs included in the study retained >30 fleas and >5 brown dog ticks at the pretreatment evaluation. Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Each run was approximately 1.2 m × 1.0 m with solid marine aluminum walls and concrete flooring. Dog runs were grouped by treatment. Each individual run was labeled with the dog identification number only and was not identified by treatment. Dogs from different treatment groups were physically separated by a solid barrier or space to prevent contact.

Dogs were fed an appropriate maintenance ration of a commercial dry canine feed (PMI Canine High Density Diet 5L18®, PMI Brentwood, MO). Water was available *ad libitum*.

2.2. Experimental design and methods

The dogs were acclimated to the study conditions for 14 days prior to treatment and were observed for general health once daily during the preconditioning period. All dogs were bathed with a non-insecticidal shampoo (Allergroom®, Studies A–C or HyLyt® Study D) on Day −13 (Studies A and B) or Day −12 (Studies C and D). During this preconditioning period, a physical exam was performed on each dog by a veterinarian to determine health and suitability for inclusion in the trial. Day 0 was the day treatments were applied to the dogs. Dogs were observed for general health and any reaction to treatment approximately 1–4 h after treatment on Day 0, then once daily for the remainder of the study.

Generally, flea and tick infestations were evaluated at 2 days after treatment and subsequently fleas were counted at 1 day after reinfestation and ticks evaluated at 2 days after reinfestation. To assess parasites, animals were examined visually and combed using a fine-toothed flea comb. For the counts, all dogs were first examined by hand and visually, and any ticks detected were removed using forceps. Ticks were examined to determine their viability. Any tick able to move in a coordinated manner was considered live. The animals were then thoroughly flea combed to count and remove fleas and any remaining ticks. Fleas able to stand upright and/or move in a coordinated manner were considered live. Flea and tick counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed before handling dogs from different groups. Personnel conducting parasite or general health exams were unaware of treatment assignments.

2.2.1. Study A

On Day −9, each dog was infested with 50 adult blacklegged (deer) ticks, *Ixodes scapularis*. On Day −8, each dog was infested with 100 unfed cat fleas, *C. felis felis*. On Day −7, each dog was thoroughly examined and combed to remove and count fleas and ticks.

The study was conducted as a randomized complete block design. For each sex there were four blocks of two animals, to provide a total of eight test animals (four of each sex) in each treatment group. Using the Day −7 flea and tick counts, eight dogs of each sex with the highest tick then flea counts were selected for inclusion in the study. For each sex, these dogs were ranked in descending order by tick count then flea count. The two dogs within each block were randomly allocated to either treatment groups A or B.

On Day −2, the dogs were each infested with 50 adult *I. scapularis*. On Day −1, the dogs were infested with 100 adult fleas each. On Day 0, each animal was treated as follows: (A) negative control, animals were not treated; (B) metaflumizone plus amitraz at the proposed commercial rate according to weight bands to provide ≥20 mg metaflumizone and 20 mg amitraz/kg (dogs from 5.1 to 9.9 kg received 1.33 ml and dogs from 10.0 to 24.9 kg received 3.33 ml of the formulation).

On Day 2, each dog was examined and combed to remove and count fleas and ticks. Subsequently, the animals were reinfested with ticks on Days 5, 12, 19, 26, 33 and 40 and with fleas on Days 6, 13, 20, 27, 34 and 41. The dogs were examined, combed and parasite counted on Days 7, 14, 21, 28, 35 and 42.

2.2.2. Study B

On Day −9, each dog was infested with 50 adult lone star ticks (*Amblyomma americanum*). On Day −7, each dog was examined and combed to remove and count ticks. The study was conducted as a randomized complete block design. For each sex there were four blocks of two animals, to provide a total of eight test animals (four of each sex) in each treatment group. Using the Day −7 tick counts, eight dogs of each sex with the highest tick then flea counts were selected for inclusion in the study. For each sex, these dogs were ranked in descending order by tick count (ascending animal identification was used to rank dogs with equal tick counts). The two dogs within each block were randomly allocated to either treatment groups A or B.

On Day −2, the dogs were each infested with 50 adult lone star ticks. On Day 0, each animal was treated as follows: (A) negative control, animals were not treated; (B) metaflumizone plus amitraz at the proposed commercial rate according to weight bands to provide ≥20 mg metaflumizone and 20 mg amitraz/kg (dogs from 10.6 to 25.5 kg received 3.4 ml of the formulation).

On Day 2, each dog was examined and combed to remove and count ticks. Subsequently, the animals were reinfested with ticks on Days 5, 12, 19, 26, 33 and 40. The dogs were examined, combed and parasite counted on Days 7, 14, 21, 28, 35 and 42. No fleas were infested in this study.

2.2.3. Study C

On Day −7, each dog was infested with 50 adult brown dog ticks, *Rhipicephalus sanguineus*. On Day −6, each dog was infested with 100 unfed cat fleas. On Day −5, each dog was thoroughly examined and combed to remove and count fleas and ticks.

The study was conducted as a randomized complete block design. For each sex there were four blocks of four animals, to provide a total of eight test animals (four of each sex) in each treatment group. Using the Day −5 flea and tick counts, 16 dogs of each sex with the highest tick and/or flea counts were selected for inclusion in the study. For each sex, these dogs were ranked in descending order by flea count (ascending animal identification was used to rank dogs with equal flea counts). The two dogs within each block were randomly allocated to either treatment groups A–D.

On Day −2, the dogs were each infested with 50 adult brown dog ticks and 50 adult American dog ticks (*Dermacentor variabilis*). On Day −1, the dogs were infested with 100 adult fleas each. On Day 0, each animal was treated as follows: (A) negative control, animals were not treated; (B) placebo control, placebo formulation at the proposed commercial dose rate for the metaflumizone plus amitraz; (C) metaflumizone plus amitraz at the proposed commercial rate according to weight bands to provide ≥20 mg metaflumizone and 20 mg amitraz/kg (dogs from 4.6 to 10.5 kg received 1.4 ml and dogs from 10.6 to 25.5 kg received 3.4 ml of the formulation); (D) positive control, fipronil plus S-methoprene given in 2.4 at the commercial dose rate.

On Day 1 (approximately 24 h after treatment), all dogs were examined and a ‘‘finger count’’ conducted to qualitatively assess the parasite burden; the dogs were examined using the hands and a comb to part the hair and expose the skin and the numbers of live ticks and fleas observed were recorded. On Day 2, each dog was examined and combed to remove and count fleas and ticks. Subsequently, the animals were reinfested with ticks on Days 5, 12, 19, 26, 33 and 40 and with fleas on Days 6, 13, 20, 27, 34 and 41. The dogs were examined, combed and parasite counted on Days 7, 14, 21, 28, 35 and 42.

2.2.4. Study D

On Day −7, each dog was infested with 50 adult brown dog ticks, *R. sanguineus*. On Day −6, each dog was infested with 100 unfed cat fleas. On Day −5, each dog was thoroughly examined and combed to remove and count fleas and ticks.

The study was conducted as a randomized complete block design. For each sex there were four blocks of two animals, to provide a total of eight test animals (four of each sex) in each treatment group. Using the Day −5 flea and tick counts, eight dogs of each sex with the highest flea counts were selected for inclusion in the study. For each sex, these dogs were ranked in descending order by flea count (ascending animal identification was used to rank dogs with equal flea counts). The two dogs within each block were randomly allocated to either treatment groups A or B.

On Day −2, the dogs were each infested with 50 adult brown dog ticks and 50 adult American dog ticks. On Day 1, the dogs were infested with 75 adult fleas each. On Day 0, each animal was treated as follows: (A) negative control, animals were not treated; (B) metaflumizone plus amitraz at the proposed commercial rate according to weight bands to provide ≥20 mg metaflumizone and ≥20 mg amitraz/kg (dogs from 4.6 to 10.5 kg received 1.4 ml and dogs from 10.6 to 25.5 kg received 3.4 ml of the formulation).

On Day 2, each dog was examined and combed to remove and count fleas and ticks. Subsequently, the animals were reinfested with ticks on Days 5, 12, 19, 26, 33 and 40 and with fleas on Days 6, 13, 20, 27, 34 and 41. The dogs were examined, combed and parasite counted on Days 7, 14, 21, 28, 35 and 42.

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2.3. Parasites

2.3.1. Study A

Cat fleas were from the NEF colony, which was initiated with fleas originally obtained from stray animals at the Stillwater Animal Shelter, Payne County, OK and had been held in culture for six generations. Blacklegged ticks were from the NEF colony, which was initiated with ticks originally obtained from deer at Payne County, OK and had been held in culture for one generation.

At each infestation, either 50 unfed adult ticks or approximately 100 unfed adult fleas were applied to each dog. The fleas or ticks were applied to the lateral midline of each dog, which was restrained by hand for sufficient time to allow the parasites to penetrate the hair coat. For tick infestations, dogs were held in tick infestation chambers (solid plastic dog crates with wire grate door) for about 8 h after ticks were applied to increase the chances of successful tick attachment.

2.3.2. Study B

Lone star ticks were from the NEF colony, which was initiated with ticks originally obtained from animals at Stillwater City pound in February 2004 and had been held in culture for one generation. Infestation methods were as for Study A.

2.3.3. Study C

Cat fleas were from the ARC colony, which was initiated with fleas originally obtained locally in Arkansas and had been held in culture. The last infusion with wild-caught fleas from a local source was about 2 weeks prior to the start of the study. Brown dog ticks and American dog ticks were obtained from EL Labs, Soquel, CA which has maintained closed colonies of ticks for over 20 years without the introduction of new ticks. American dog ticks were also obtained from Oklahoma State University for the Day 33 infestation. This colony has been maintained for over 30 years, originating from ticks collected in Stillwater, OK, with wild-caught ticks introduced into the colony every 2 years.

At each infestation, either 50 unfed adult ticks of each species or approximately 100 unfed adult fleas were applied to each dog. The fleas or ticks were applied to the lateral midline of each dog, which was restrained by hand for sufficient time to allow the parasites to penetrate the hair coat.

2.3.4. Study D

Cat fleas were from the STM colony, which was initiated with fleas originally obtained from Kansas State University in 1992. Since 1998, additional fleas from the EL Labs (Soquel, CA) colony have been introduced annually. Brown dog ticks were obtained from a culture maintained for about 13 years by Professional Laboratories (PLRS, Corapeake, NC), originally obtained locally with additional introductions of ticks from the EL Labs colony every 3–4 years. American dog ticks were obtained from Oklahoma State University. This colony has been maintained for over 30 years, originating from ticks collected in Stillwater, OK, with wild-caught ticks introduced into the colony every 2 years.

At each infestation, either 50 unfed adult ticks of each species or approximately 100 unfed adult fleas were applied to each dog. The fleas or ticks were applied to the lateral midline of each dog, which was restrained by hand for sufficient time to allow the parasites to penetrate the hair coat.

2.4. Treatment

The animals receiving metaflumizone plus amitraz were treated with a commercial formulation containing 150 mg metaflumizone and 150 mg amitraz/ml. The placebo-treated dogs (Study C) were treated with the same solvent formulation with out the active ingredients. All applications were administered using disposable syringes. Doses were calculated using pretreatment body weights and were based on the proposed doses for weight bands. The positive control dogs (Study C) were treated with the commercial fipronil plus S-methoprene formulation, Frontline Plus® for Dogs and Puppies (Merial, Duluth, GA). The doses were applied to the skin at a single spot between the shoulder blades.

2.5. Data analysis

Statistical analyses were performed separately for flea and tick counts and for each examination day. Flea and tick counts were transformed by the $\log_{10}$(count + 1) transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 8.2, Cary, NC), transformed counts were analyzed by an analysis of variance (ANOVA) with a model that considered treatment as a fixed effect and replicate as a random effect. Treatment was tested against the residual error at the 5% level of significance. The least square means (LSMeans) were calculated for each group. Since there were only two treatment groups in Studies A, B and D the ANOVA F-test was used to determine the treatment

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Table 1
Geometric mean flea counts and percent efficacy[a] relative to nontreated controls for Beagles (Study A) and mixed breed dogs (Study D) treated with metaflumizone plus amitraz

Count day	Study A		Study D	
	Nontreated control	Metaflumizone plus amitraz	Nontreated control	Metaflumizone plus amitraz
−7/−5	73.2	75.8	65.9	65.1
2	70.4	0.5* (99.3)	55.9	0.0* (100)
7	73.5	0.8* (98.9)	52.1	0.1* (99.8)
14	70.8	0.5* (99.3)	92.9	0.0* (100)
21	75.3	0.3* (99.7)	88.7	0.2* (99.8)
28	78.1	4.4* (94.4)	79.4	2.6* (96.7)
35	79.0	9.6* (87.9)	77.2	6.5* (91.6)
42	70.9	15.9* (77.6)	76.7	8.0* (89.6)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

effect. Half of the P-value from the F-test was used to provide the one-sided test used to determine if the reduction in parasite count of the treated group relative to the control group was significant at the 5% level. In Study C, each treatment group's LSMean was compared with the nontreated control using the one-sided Student's t-test at the 5% level of significance.

Percent efficacy, relative to the nontreated control group and based on geometric means (gm), was calculated as follows:

$$\text{efficacy}\,(\%) = \frac{\text{gm control} - \text{gm treated}}{\text{gm control}} \times 100$$

3. Results

3.1. Fleas

All dogs included in the studies demonstrated good pretreatment flea holding ability. Mean pretreatment flea counts ranged from 65 to 76 across the three studies (Tables 1 and 2). Nontreated animals maintained flea infestations through out the studies. The placebo treatment had no detrimental effect on flea numbers (Table 2). There was no significant difference between nontreated and placebo-treated mean counts on any day ($P < 0.05$) and mean counts were numerically higher for placebo-treated animals than for the nontreated controls on every day.

The metaflumizone plus amitraz treatments resulted in significantly lower flea counts relative to nontreated controls on all posttreatment count days ($P < 0.05$, Tables 1 and 2). The Day 1 finger counts in Study C showed that treatment with metaflumizone plus amitraz gave rapid control of existing flea infestation with a reduction of 99.4% within 24 h after treatment. Metaflumizone plus amitraz provided at least 99% control of fleas from Days 2 to 21 in all three studies, and >90% reduction to Day 28 (Study A) or Day 35 (Studies C and D). Flea control for metaflumizone plus amitraz at Day 42 ranged from 77.6 to 89.6% for the three studies. This level of flea control was similar to that provided by fipronil plus methoprene (Table 2).

Table 2
Geometric mean flea counts for nontreated and placebo control dogs, and counts and percent efficacy[a] relative to nontreated controls for mixed breed dogs treated with metaflumizone plus amitraz or fipronil plus S-methoprene (Study C)

Count day	Nontreated control	Placebo control	Metaflumizone plus amitraz	Fipronil plus S-methoprene
−5	70.8	71.8	70.3	70.4
1	43.7	67.0	0.3* (99.4)	0.0* (100)
2	53.3	81.6	0.0* (100)	0.0* (100)
7	62.4	84.1	0.0* (100)	0.0* (100)
14	64.2	94.8	0.3* (99.6)	0.0* (100)
21	68.5	96.6	0.0* (100)	0.0* (100)
28	55.8	75.1	0.6* (98.8)	0.0* (100)
35	56.9	72.3	2.3* (95.9)	0.1* (99.8)
42	49.7	91.4	6.6* (86.6)	0.4* (99.3)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

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Table 3
Geometric mean *Ixodes scapularis* (blacklegged tick, Study A) and *Amblyomma americanum* (lone star tick, Study B) counts and percent efficacy[a] relative to nontreated controls for Beagles treated with metaflumizone plus amitraz

Count day	*Ixodes scapularis*		*Amblyomma americanum*	
	Nontreated control	Metaflumizone plus amitraz	Nontreated control	Metaflumizone plus amitraz
−7	19.4	20.1	17.6	17.2
2	17.7	0.9* (94.8)	17.8	2.8* (84.3)
7	15.4	0.6* (96.0)	18.3	1.0* (94.8)
14	14.0	0.7* (94.9)	19.3	1.0* (95.0)
21	13.0	1.2* (91.0)	16.4	1.0* (93.7)
28	15.9	2.9* (82.0)	17.0	4.1* (75.6)
35	20.3	4.2* (79.4)	19.0	1.3* (93.0)
42	17.3	5.1* (70.8)	19.7	5.0* (74.9)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

3.2. Ticks

3.2.1. *Ixodes scapularis*

All dogs demonstrated good pretreatment tick holding ability. Day −7 tick counts ranged from 12 to 30. Nontreated animals maintained tick infestations through out the study (Table 3). The metaflumizone plus amitraz treatment resulted in significantly lower tick counts relative to nontreated controls on all post-treatment count days ($P < 0.05$, Table 3). Treatment resulted in about 95% control of an existing infestation within 48 h of treatment. Following weekly reinfestations, control was >90% to Day 21, 82% on Day 28, and about 79 and 71% on Days 35 and 42, respectively.

3.2.2. *Amblyomma americanum*

Day −7 tick counts ranged from 12 to 24, demonstrating good pretreatment tick holding ability for all the dogs. Nontreated animals maintained consistent tick infestations through out the study (Table 3). Treatment with metaflumizone plus amitraz resulted in significantly lower tick counts relative to nontreated controls on all post-treatment count days ($P < 0.05$, Table 3). Treatment resulted in 84.3% efficacy against an existing infestation within 48 h of treatment. Following weekly reinfestations, control was >93% to Day 21, 75.6% on Day 28, and 93.0 and 74.9% on Days 35 and 42, respectively.

3.2.3. *Rhipicephalus sanguineus*

All dogs included in the studies demonstrated good pretreatment tick holding ability. Day −5 brown dog tick counts ranged from 6 to 47 (Study C) and 26 to 50 (Study D). Nontreated animals maintained tick infestations throughout the study (Tables 4 and 6). The placebo treatment appeared to have little effect on geometric mean brown dog tick numbers (Table 4). Though mean tick numbers were significantly lower than nontreated control counts on Days 1, 2, and 7 ($P < 0.05$); the maximum reduction was 52.7% on Day 7 and the pretreatment mean for the placebo group was about 30% lower than that for the nontreated animals. In addition, the mean number of brown dog ticks recovered from the placebo dogs on Day 2 was similar to the pretreatment count for this group (Table 4). Treatment with metaflumizone plus amitraz resulted in significantly lower tick

Table 4
Geometric mean *Rhipicephalus sanguineus* (brown dog tick) counts for nontreated and placebo control dogs, and counts and percent efficacy[a] relative to nontreated controls for mixed breed dogs treated with metaflumizone plus amitraz or fipronil plus S-methoprene (Study C)

Count day	Nontreated control	Placebo control	Metaflumizone plus amitraz	Fipronil plus S-methoprene
−5	29.6	20.7	22.2	27.7
1	24.1	13.4*	0.6* (97.4)	0.2* (99.2)
2	30.9	18.8*	0.1* (99.7)	0.0* (100)
7	23.2	11.0*	0.0* (100)	0.0* (100)
14	23.4	19.7	0.0* (100)	0.0* (100)
21	27.8	16.7	1.0* (96.5)	0.1* (99.7)
28	21.8	13.7	2.9* (86.5)	0.3* (98.6)
35	15.1	13.6	9.9 (34.5)	2.5* (83.4)
42	21.2	16.0	17.5 (17.3)	3.9* (81.8)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

Table 5
Geometric mean *Dermacentor variabilis* (American dog tick) counts for nontreated and placebo control dogs, and counts and percent efficacy[a] relative to nontreated controls for mixed breed dogs treated with metaflumizone plus amitraz or fipronil plus S-methoprene (Study C)

Count day	Nontreated control	Placebo control	Metaflumizone plus amitraz	Fipronil plus S-methoprene
1	17.4	18.5	0.4* (97.6)	0.4* (97.5)
2	20.9	18.3	0.0*(100)	0.0* (100)
7	29.4	28.6	0.0*(100)	0.0* (100)
14	33.4	27.9	0.0*(100)	0.0* (100)
21	27.8	27.1	1.0* (96.3)	0.0* (100)
28	30.5	26.8	3.1* (89.8)	1.9* (93.8)
35	26.5	28.6	15.0 (43.3)	7.9* (70.1)
42	24.7	22.4	19.4 (21.8)	12.8 (48.4)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

counts relative to nontreated controls to 28 days after treatment in Study C ($P < 0.05$, Table 4) and Day 35 in Study D (Table 6). The finger counts on Day 1 (Table 4) indicated that the metaflumizone plus amitraz treatment resulted in >97% control of brown dog ticks within 24 h of treatment. Following weekly reinfestations, control in the two studies was >95% to Day 21, >85% on Day 28, and about 35% (Study C) or about 80% (Study D) on Day 35, efficacy declined further on Day 42 (Tables 4 and 6). The brown dog tick control provided by treatment with metaflumizone plus amitraz was similar to that provided by the positive control up to Day 28 (Table 4).

3.2.4. *Dermacentor variabilis*

Nontreated animals maintained American dog tick infestations through out the study (Tables 5 and 6). The placebo treatment appeared to have no effect on American dog tick numbers (Table 5). There was no significant difference between nontreated and placebo-treated mean counts on any day ($P > 0.05$); and mean counts for both groups were similar on all days. Treatment with metaflumizone plus amitraz resulted in significantly lower American dog tick counts relative to nontreated controls to 28 days after treatment in Study C ($P < 0.05$, Table 5) and Day 42 in Study D (Table 6). Finger counts on Day 1 (Table 5) indicated that the metaflumizone plus amitraz treatment resulted in >97% control of American dog ticks within 24 h of treatment. Following weekly reinfestations, control in the two studies was >95% to Day 21, >89% on Day 28, and about 43% (Study C) or about 84% (Study D) on Day 35, efficacy declined further on Day 42 (Tables 5 and 6). The control of American dog tick provided by treatment with metaflumizone plus amitraz was similar to that provided by the positive control up to Day 35 (Table 5).

3.3. Health observations

3.3.1. Study A

One nontreated dog was noted to have produced a loose stool at about 2 h after treatment on Day 0, but was noted as normal thereafter. There were no other adverse health observations noted during the study.

3.3.2. Study B

One nontreated dog was diagnosed with lumbar disc disease (slipped disc, possibly caused by the animal slipping while confined in the tick infestation chamber)

Table 6
Geometric mean *Rhipicephalus sanguineus* (brown dog tick) and *Dermacentor variabilis* (American dog tick) counts and percent efficacy[a] relative to nontreated controls for Beagles treated with metaflumizone plus amitraz (Study D)

Count day	*Rhipicephalus sanguineus*		*Dermacentor variabilis*	
	Nontreated control	Metaflumizone plus amitraz	Nontreated control	Metaflumizone plus amitraz
−5	37.9	40.5	(−)	(−)
2	35.0	0.0* (100)	34.2	0.0* (100)
7	27.5	0.0* (100)	27.5	0.0* (100)
14	36.6	0.0* (100)	35.3	0.0* (100)
21	39.1	0.1* (99.8)	40.5	0.3* (99.4)
28	29.1	3.5* (88.1)	39.1	4.1* (89.4)
35	29.6	6.2* (79.0)	37.6	6.0* (83.9)
42	24.8	11.0 (55.7)	19.7	5.0* (74.9)

[a] Geometric mean counts with * are significantly different to nontreated control; $\alpha = 0.05$. Percent efficacy is given in brackets.

on Day −1. The dog was treated with a nonsteroidal anti-inflammatory drug and responded well to treatment. The dog was able to stand without assistance by Day 1, and progressively improved and was considered normal by Day 38. While this animal had restricted mobility for a number of days this did not affect tick infestation and did not adversely impact the study. One treated animal was noted to have produced one normal stool and one loose stool when inspected at about 1 h after treatment on Day 0, but was noted as normal at all subsequent observations.

3.3.3. Study C

A number of dogs were noted with minor ailments during the study. Occasional transitory emesis and loose stools were noted in a few dogs from each group. There was no apparent difference in incidence among the groups. One metaflumizone plus amitraz-treated dog was noted with tremors on Day 11. Further examination of this animal determined that this was a nervous behavior; tremors were not apparent prior to approaching the dog and dissipated after handling the animal. A fipronil plus S-methoprene-treated dog was observed with a small subcutaneous swelling on the left front leg on Days 20–22. On each day, the swelling was pierced and drained; the swelling was reduced in size on Day 23 and no further treatment was required. One placebo-treated dog was noted with "hot spots" on the rump, possibly fleabite dermatitis, on Day 21. These spots were cleaned with peroxide and/or treated with an antibiotic ointment daily until Day 42. Except where noted, none of these minor ailments required treatment.

3.3.4. Study D

One dog in the metaflumizone plus amitraz group was noted with a lesion on the back, possibly flea bite dermatitis, prior to treatment. The dog was treated with an oral anti-inflammatory for 7 days. The lesion was noted to be healing by Day 5. A nontreated dog vomited while being combed on Day 14, but was otherwise reported as normal when observed subsequently. A few dogs were noted with mild alopecia. Except where noted, none of these minor ailments required treatment.

4. Discussion

4.1. Fleas

There were no significant differences in parasite counts between the nontreated control and the placebo-treated control group for fleas on any post-treatment count day. These results in mixed breed dogs confirmed observations in a previous study with Beagles that the formulation excipients have no antiparasitic or repellent activity against fleas (Rugg and Hair, this volume).

Treatment with the metaflumizone plus amitraz formulation at the proposed commercial dose rate provided significant reductions in flea counts relative to nontreated dogs for at least 42 days after treatment for three different flea strains in both Beagles and mixed breed dogs. The rapid effect of treatment on an existing flea infestation was demonstrated in one study where a finger count showed that efficacy at 24 h after treatment was >99%. In all three studies, treatment with metaflumizone plus amitraz resulted in >99% control of fleas within 24 h following infestation up to Day 21, and >90% control to Days 28 or 35. At study end (Day 42), flea control ranged from 77.6 to 89.6% for the three studies. This level of flea control was similar to that provided by the positive control product and indicates that treatment with metaflumizone plus amitraz can provide flea control for up to 6 weeks after treatment and use as a monthly treatment will provide highly effective control of fleas on dogs.

Newly infested female fleas cannot lay eggs for the first 24–36 h and require a blood meal to produce viable eggs (Rust and Dryden, 1997). The rapid kill of fleas especially over the initial month post-treatment means that infesting fleas will be killed prior being able to produce eggs. In addition, the mode of action of metaflumizone (sodium channel blocker) results in the rapid cessation of feeding as one of the first symptoms of poisoning in insects (Wing et al., 1998; Salgado and Hayashi, this volume). Thus it is likely that treatment with metaflumizone plus amitraz will also prevent the reinfestation of animals by breaking the life cycle of the flea through the prevention of egg laying as has been demonstrated with other on animal adulticide products (Hutchinson et al., 1998; Dryden et al., 2000; Shanks et al., 2000).

4.2. Ticks

The placebo-treatment had little if any effect on the two common dog ticks, *R. sanguineus* and *D. variabilis*. Though *R. sanguineus* counts were significantly lower for the placebo-treated dogs ($P < 0.05$) for the first week after treatment; maximum reduction was about 53% and pretreatment counts for the placebo group were 30% lower. For *D. variabilis* counts were always similar and not significantly different to nontreated animals ($P > 0.05$). These results in mixed breed dogs confirmed the results seen with *R. sanguineus* in Beagles (Rugg and Hair, this volume) that the formulation excipients had little if any effect on tick infestations.

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Treatment with metaflumizone plus amitraz at the proposed commercial dose rate resulted in rapid control of existing infestations of *R. sanguineus* and *D. variabilis*. Counts of both species were reduced by >97% within 24 h of treatment relative to nontreated animals ($P < 0.05$). Treatment with metaflumizone plus amitraz provided significant reductions in tick counts relative to nontreated dogs for 28–42 days after treatment for the four different tick species. Efficacy against all species was >90% to Day 21 and ranged from 75.6 (*A. americanum*) to 89.8% (*D. variabilis*) on Day 28. Efficacy generally tended to decline thereafter, though efficacy against *A. americanum* was 93.0% on Day 35. In Study C, tick control provided by metaflumizone plus amitraz was generally similar to that provided by the positive control for at least 4 weeks after treatment. These studies demonstrated that metaflumizone plus amitraz applied at the proposed commercial dose rate will provide rapid control of existing tick infestation and provide at least 4 weeks residual protection against infestation by the common tick species affecting dogs.

4.3. Health observations

The minor ailments such as transient diarrhea or emesis seen in some dogs (including nontreated and placebo-treated dogs) are not uncommon in confined dogs (especially when animals are nervous or agitated) and were unlikely to have been related to the treatment. There were no apparent adverse reactions to treatment.

5. Conclusions

These studies confirmed that treatment with metaflumizone plus amitraz at the proposed commercial dose rate rapidly controlled existing infestations of fleas and the common tick species found on dogs. Treatment provided control of reinfesting fleas for up to 6 weeks and at least 4 weeks control of ticks. Efficacy was confirmed in a variety of dog breeds against three different fleas strains and four common species of ticks found on dogs in the United States.

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