A Comparison of Flea Control Measurement Methods for Tracking Flea Populations in Highly Infested Private Residences in Tampa FL, Following Topical Treatment of Pets With Frontline® Plus (Fipronil/(S)-Methoprene)

Michael Dryden[a]
Doug Carithers[b]
Amy McBride[a]
Breanne Riggs[a]
Lindsey Smith[a]
Jacob Davenport[a]
Vicki Smith[a]
Patricia Payne[a]
Sheila J. Gross[c]

[a] *Kansas State University CVM, 1800 Denison Avenue, Manhattan, KS 66506*
[b] *Merial Limited, 3239 Satellite Boulevard, Duluth, GA 30096*
[c] *Independent Statistician, 26 Bayberry Close, Piscataway, NJ 08854*

KEY WORDS: *Ctenocephalides felis*, fleas, emergent fleas, flea sex ratio, dog, cat, household study, flea trap counts, on-animal flea counts

ABSTRACT

The efficacy of fipronil/(S)-methoprene (FRONTLINE® Plus, Merial Limited, Duluth, GA, USA) for household flea control was assessed against existing natural flea populations in homes in Tampa, Florida. Twenty-seven households fulfilled requirements for inclusion into and completed this non-randomized, multiple site (household), prospective field study. The study duration was 60 days, with all household dogs and cats treated with FRONTLINE Plus on day 0 and day 28-30. Three separate household flea-control assessment methods were used in this study, and each was evaluated to determine its ability to effectively gauge household flea control of natural flea populations in homes. The three methods of assessing household flea control were: 1) Counting fleas captured in intermittent-light environmental flea emergence traps placed in heavily infested areas of the house. 2) Examination of the sex ratios of the fleas captured in the intermittent-light environmental flea emergence traps, and 3) assessing pet flea burdens, as has been typically done historically. Our findings in this study demonstrated that in field situations, the most accurate measure of household flea

control trends is determined by comparing counts, over time, of newly emerged (unfed) fleas caught in intermittent-light environmental flea emergence traps. This study also verified that determining sex ratios of the newly emerged trapped fleas provides the best indication of the immediate population trend in flea-infested households. In household studies such as this, when household pets have access to the outdoor environment, this study clearly demonstrated that on-animal flea counts can be inconsistent and provides the least reliable indicator available for measuring suppression of household flea infestations. These findings demonstrated that in field situations FRONTLINE Plus provided effective flea control, as assessed by the significant decline in flea emergence from the contaminated household.

®FRONTLINE is a registered trademark of Merial in the United States of America and elsewhere.

INTRODUCTION

Optimal conditions for development of *Ctenocephalides felis* are relative humidity >70% and temperatures between 20^0 and 30^0C.1 The summertime temperature and humidity in Tampa, Florida, typically provide near optimal conditions for flea development, and large natural flea burdens were seen in previous studies conducted during summers in the Tampa area.[2,3,4,5] It was determined that this would provide an excellent study location to compare flea control assessment methods against a heavy natural flea challenge, using FRONTLINE Plus (Merial Limited, Duluth, GA).

Intermittent-light flea traps and pet area flea counts have been used in previous studies to evaluate elimination of household flea populations. The intermittent-light flea traps are proven to be highly effective for collecting newly emerged fleas. The flea trap design used in these studies has been shown to collect >86% of live fleas released into a carpeted 10.23 m^2 room (3.1 x 3.3 m) during a 20-h test period.[6] In previous in-home investigations, it was noted that following treatment of all dogs and cats in some households, the environmental flea emergence trap counts increased within 1 to 3 weeks following treatment.[7] Considering the length of the cat flea life cycle, these increased numbers of newly emerged fleas originated from pre-existing (pre-treatment) immature flea life-stages within the home premises.[7,8]

In some cases, the increase has been so dramatic (20% or greater increase in counts) that these households have been referred to by the author as "red-line homes," indicating that at the time of treatment, the flea population was either in a rapid growth phase, or development and emergence was initially delayed by environmental conditions (ie: cool ambient temperatures). Therefore, the eventual reduction in newly emerged fleas captured in traps reflects exhaustion of the pre-existing infestation and prevention of new, viable flea egg production.[2,3,4,5]

A second measure of flea population evaluation also relied upon assessment of the trapped fleas. The sex ratios of the captured newly emerged unfed fleas in these homes were assessed to determine if sex ratios might be used as an immediate predictor of household flea control status. While most insect species exhibit protandry (males tending to emerge before females), *C. felis* belong to a much smaller group that exhibits protogyny (females tend to develop before males).[8] The first fleas to emerge from a single, time-delineated, cohort of eggs are female fleas, followed by both males and females, and then lastly, almost exclusively males. Therefore, in an infestation with active reproduction, a predominance of female fleas should be sustained throughout. However, when reproduction has ceased in a flea population, due either to natural causes or to treatment intervention (ie, killing fleas before they reproduce or preventing flea development), a population shift from a predominantly female flea to predominantly male fleas would be observed.

The third measure evaluated for assessing household flea population dynamics was the use of on-animal flea population

estimates. This assessment has historically been used to measure household flea control achieved with flea control products. [2,3,4,5] The consistency of on-animal assessment results in the home environment were then compared to the other two measures of household flea population dynamics.

MATERIALS AND METHODS

Study Procedure

This was a non-randomized, multiple-site (household), prospective field study for evaluation of flea emergence, control and flea sex ratios in heavily infested home environments in the Tampa area, where all pets in the household were treated with FRONTLINE Plus. Each household was considered an experimental unit for site assessments (flea emergence, sex ratio assessments), and each animal was considered an experimental unit for on-animal flea assessments.

All animals were client-owned dogs and cats and were handled in compliance with Kansas State Institutional Animal Care and Use Committee (IACUC) and Merial IACUC approval. Throughout the trial, dogs and cats were housed in their normal environment. Qualifying households for enrollment were gathered via referrals from The Sunshine Animal Hospital, Tampa, FL, and also advertisements on CRAIGSLIST®.

Twenty-nine flea-infested homes in the Tampa, FL, area met the inclusion criteria and were selected for the study, which was performed during the late spring and summer of 2009. Homes and pets were selected based on the following inclusion criteria:

1. Fleas present on pets (minimum of five fleas in total area counts on at least one animal), animals with at least five fleas upon initial exam included in the study.
2. Fleas present in the home (based upon at least five newly-emerged fleas captured in intermittent-light flea traps).
3. One to four healthy dogs or cats residing at the residence.
4. Owners willing to participate in the study for at least 2 months.
5. Owners willing to not use any other topical or premise flea control products during the study.
6. Owners understood there were no restrictions on the animals regarding exposure to rain, swimming, or movement outdoors.
7. No mammalian pets other than dogs or cats were present in household.

®CRAIGSLIST is a registered trademark of Craigslist, Inc. in the United States of America and elsewhere.

Additionally, prior to official enrollment, all pet owners signed a consent form and completed a questionnaire on pet health, flea control product use, history of flea infestations on pets, description of the indoor & outdoor activity of each of the pets, and questions about the home's yard, including information on wildlife and feral cats observed in the yard and neighborhood.

Treatments

All pets in each enrolled household were treated on Day 0 and then once again between Days 28–30. All treatments were applied by study investigators. No other topical or premise flea treatments were used during the study. While pet activity was not restricted, it was recorded.

Dogs were treated topically with fipronil (9.8% w/w)–(S)-methoprene (8.8% w/w) and cats were treated with fipronil (9.8% w/w)–(S)-methoprene (11.8% w/w) (FRONTLINE Plus, Merial) according to label dosing recommendations. The entire dose was applied in one spot between the shoulder blades for dogs or at the base of the neck for cats. Pets were weighed on Day –1 or Day 0 and again during the Day 28 – 30 site visit to ensure proper dosing and were treated by the investigators. In some homes, not all pets qualified for inclusion in the study (fewer than five fleas, inability to examine, etc). However, every dog and cat within each enrolled household was treated with FRONTLINE Plus according to weight/

species, whether each individually qualified for study inclusion or not.

Measurements

The numbers of adult fleas emerging in the home were assessed using intermittent- light premises flea emergence traps.[2,3,4,5,6] Environmental flea counts were conducted ± 1 day on Days 0, 7, 14, 21, then once between Days 28-30, 40-45, and 54-60. Site selection, for trap placement, was based on where the pet(s) typically spent most of their time or where owners had observed emerging fleas. Two traps were placed in two separate rooms/areas for 16 to 24-hr for each household assessment period. Once trap locations were selected for a household, the traps were returned to the exact same locations for each subsequent counting period to ensure an accurate assessment of flea emergence in that locale was gathered. Fleas collected on the adhesive pads of the traps were counted and characterized by microscopic observation as to species, newly emerged (or fed) and the sex of the newly emerged fleas.

As was done in previous studies, the number of fleas on each pet was estimated using a visual area count methodology.[2,3,4,5,10] Area counts were performed at five locations on each animal:

- dorsal midline
- tail head
- left lateral
- right lateral
- inguinal region.

Area counts were limited to 1 minute per location and conducted by parting the hair against the lay using both hands until the skin surface of the selected area was viewed. Animal flea count estimates were conducted ± 1 day on Days 0, 7, 14, 21, then once between Days 28-30, 40-45, and 54-60.

Data Analysis

All statistical assessments in this study were performed using SAS version 9.2. Environmental control assessments were calculated using Geometric Means (GM), for which counts were transformed to the natural logarithm of (count + 1) for calculation. Environmental control assessments using flea trap counts were calculated based on the highest counts of unfed fleas captured in traps between study Day 0 and the Day 28-30 assessments, compared to the final assessment. This was done in consideration of the length of the cat flea life cycle, as emergent fleas in that time frame could only have originated from pre-existing (pre-treatment) immature flea life-stages already present in the home.[7,8]

(Day z GM Flea trap Counts - Day y GM Flea trap Counts)/Day z GM Flea trap Counts x 100 = % control

Where z = the Day with the highest number of fleas collected within the first 30 days of enrollment, and where y = Day 54–60 (completion date of study for that household).

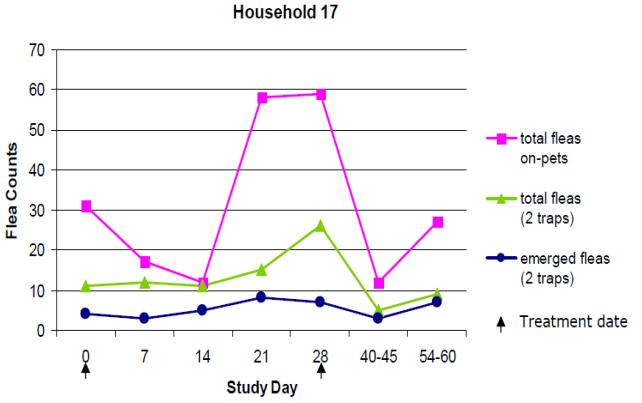
Fleas collected in traps were assessed for sex throughout the study, noting the arithmetic ratio of female to male fleas (F:M) for each study site. All flea trap count assessments were performed only including the unfed newly emerged fleas captured in the traps.

On-animal counts of live adult fleas were transformed to the natural logarithm of (count + 1) for calculation of geometric means by assessment group at each time point. Percent reduction from the control (Day 0) mean were calculated using the formula [(C - T) / C] x 100, where C = geometric mean for the control count (Day 0) count and T = geometric mean for the treated group for each subsequent assessment.

In order to compare the reductions in flea counts in traps from the maximum count to the end of the study (Day 54-60), the final value was subtracted from the starting value, and the difference was transformed to the natural logarithm of (count + 1). If the final count was higher than the initial count, causing a negative "reduction," the difference was defined as equal to zero. A t-test was then performed on the transformed differences with a null hypothesis that the mean difference was equal to zero; 2-sided p-values are reported.

To compare the sex ratio of fleas in traps

Figure 1. *Flea burden trends seen for total fleas on-pet and for newly emerged fleas in a household with limited external flea contamination sources, with monthly on-animal treatment of all dogs and/or cats in the household.*



from the maximum count to the end of the study (Day 54-60), a test of two independent proportions was used. Two-sided p-values are reported.

Flea counts on the pets were summarized for each site by calculating geometric means for each site and day as described for trap counts; maximum count for each animal or each site was not determined. Geometric means overall and for each site were calculated as described for trap counts, with each site having equal weight in the calculation, and percent reduction from Day 0 to Day 54-60 was calculated as described above for trap counts. The p-value for the difference between Day 0 and Day 54-60 flea counts was determined in the same manner as for trap counts.

RESULTS

Twenty-nine households initially qualified and were enrolled into the study. Two of the 29 households (site numbers 3 and 11) were excluded from data analysis due to protocol violations. In the 27 homes that completed the study, flea traps were placed for flea collection a total of 378 times (two sites/house/collection for seven collections). All fleas recovered in the traps were identified as C. felis (cat flea). Of the 2,241 total fleas collected in the traps, 771 (34.4%) of those fleas exhibited visible evidence of having previously fed when assessed microscopically. Of the 771 fed fleas, only nine were considered to be engorged females, indicating that they had been on a host for at least 24 hours.

In-depth investigations of the sites where these fully engorged female fleas were found confirmed that untreated animals, not on study, had access to those households just prior to or concurrently with trap placement in the home. Additionally, in all cases where partially fed fleas were identified in traps near the end of the study, site investigations identified areas in the yard acting as sources of flea contamination for the indoor/outdoor pets in the household. Flea population results seen at a household with documented visitor pets are shown in Figure 1, while

Table 1. *Intermittent Light Flea Trap counts of newly emerged unfed Ctenocephalides felis felis*

	Day								
	0[a]	7	14	21	28-30	40-45	54-60	% reduction from Day 0	% reduction from maximum trap count
All Homes	6.7	3.8	6.3	4.9	2.4	2.4	1	85.4%*	92.5%*
-red line homes[b]	5.8	5.6	12	7.9	3	4.3	1.7	69.7%*	90.9%*
-non-red-line homes	7.6	2.7	3.7	3.3	2	1.4	0.5	93.1%*	94.5%*

[a] *Day 0 was not the same for all homes (Range: 19-M-2009 to 9-June 2009)*
[b] *Red line home- homes where the flea trap counts increased by ≥ 20% within initial 30 days following treatment*
** Reduction observed was statistically significant ($p<0.05$)*

Table 2. *Intermittent Light Flea Trap counts of newly emerged unfed Ctenocephalides felis felis, by percent (%) female*

	Day						
	0[a]	7	14	21	28-30	40-45	54-60
All Homes	57.50%	59.30%	44.70%	32.20%	41.40%	40.00%	25.0%*
-red line homes[b]	65.80%	62.00%	42.50%	28.80%	42.00%	41.20%	21.4%*
-non-red-line homes	53.40%	53.80%	51.30%	39.00%	40.80%	30.80%	33.3%*

[a]Day 0 was not the same for all homes (Range: 19-M-2009 to 9-June 2009)
[b] Red line home- homes where the flea trap counts increased by ≥ 20% within initial 30 days following treatment
**Reduction observed from Day 0 to Day 54-60, was statistically significant (p< 0.05)*

Table 3. *Intermittent Light Flea Trap counts of newly emerged unfed Ctenocephalides felis felis, by gender ratio: Female to Male*

	Day						
	0[a]	7	14	21	28-30	40-45	54-60
All Homes	1.35:1	1.46:1	1:1.2	1:2.1	1:1.4	1:1.5	1:3.0
-red line homes[b]	1.92:1	1.63:1	1:1.4	1:2.5	1:1.4	1:1.4	1:3.7
-non-red-line homes	1.15:1	1.16:1	1.05:1	1:1.6	1:1.4	1:2.2	1:2.0

[a]Day 0 was not the same for all homes (Range: 19-M-2009 to 9-June 2009)
[b] Red line home- homes where the flea trap counts increased by ≥ 20% within initial 30 days following treatment
**Reduction observed from Day 0 to Day 54-60, was statistically significant (p< 0.05)*

Figures 2 and 3 illustrate flea population findings at sites with known outdoor infestation sources.

Thus, the average total number of fleas recovered per trap per collection was 5.93 fleas, but when one considered only the newly emerged unfed fleas, the average newly emerged flea number was 3.88 recovered per assessment. For the final assessments of household environmental control and sex ratios, only data from the newly emerged unfed fleas captured in flea traps were used.

Two patterns of flea trap counts were observed in the 27 homes during the study period. In 15 homes, the number of newly emerged (unfed) fleas caught in emergence traps progressively declined from Day 0 to the conclusion of the study. In 12 homes (44.4% of the households), however, flea trap counts increased by >20% after Day 0, with the highest counts typically seen on study Days 7, 14, and/or 21 (redline homes). Thereafter, flea trap counts progressively declined.

When the trap reductions were measured against the highest measured unfed flea trap count within in the first 30 days, the percent reduction in flea trap counts for all combined households was 92.5%, red-line homes saw a 90.9% reduction, and the non red-line homes reduced by 94.5% (Table 1). Again, at all time points, reductions in flea trap counts were statistically significant ($p<0.05$).

In all homes, at and near initiation of the study, most newly emerged fleas in the intermittent-light flea traps were female, representing 57.5% (Day 0) to 59.3% (Day 7) (F:M=1.4:1) of the captured flea population (Tables 2 and 3). By the end of the study, the number of unfed female fleas represented only 25% (F:M = 1:3) of the captured flea population, demonstrating that a significant shift in the sex ratio ($P<0.05$) had occurred during the study period. In this study, the shift in sex ratio was most pronounced in the red-line homes, with female fleas representing 62%-65.8% initially (1.9:1), then falling to 21.4% by study end (1:3.7, $p<0.05$).

For on-animal pet area flea counts performed on study Day 0, the geometric mean number of fleas was 17.1 ± 0.77 with no difference between flea counts on pets in red-

line and non red-line homes. Since the area flea count methodology detects an average of 23.5% of the total on-animal flea burden, the initial mean flea burden on these pets was estimated to be approximately 73 fleas per pet.[9] By the end of the study (Day 54-60), on-animal flea counts from all households declined 87.5% from Day 0 ($p<0.05$). The percent reduction in flea counts for pets in red-line homes was 69.7% ($p<0.05$), and for non -red-line homes there was a 95.0% reduction of on-animal flea counts ($p<0.05$).

In most of the non red-line homes, flea burdens on the pets and flea trap counts both declined and continued to do so throughout the study (Figure 4). While in red-line homes, on-animal flea counts typically initially decreased following treatment, and then increased dramatically, coinciding with the increased emergence of fleas from pre-existing pre-adults stages in the environment (Figure 5).

It is important to note that the trends of trap count reduction and sex ratio population shift (from predominantly female to male populations) of unfed fleas were consistent through the study. However, the on-pet flea counts did not always coincide with these trap count and sex ratio trends and continued to fluctuate throughout the study.

DISCUSSION

Knowledge of the flea lifecycle, existing premises contamination, and of flea population kinetics, including a comprehension of the effects of temperature and humidity on flea populations is critical for appropriately managing homeowner expectations for the control of flea infestations. This becomes apparent when one considers fed vs unfed fleas in light traps. Intermittent-light environmental flea emergence traps were designed, and have been demonstrated, to be highly effective in attracting newly emerged unfed fleas.[6] When emergent flea traps are placed in the same location over time, the unfed fleas captured in traps allow an accurate assessment of local flea emergence, thus the ability to determine the viable household contamination. This is possible because the cat flea is considered to be a permanent ectoparasite and only sustains egg production while on the host.[11]

In this study, we found that one-third of the fleas in the traps had previously fed, indicating that they had fed on humans or pets and were not unfed fleas. Determination that a blood meal had been taken was recognizable microscopically by the presence of blood in the midgut of the flea, or a droplet of blood deposited on the sticky adhesive of the flea trap immediately posterior to the flea, indicating the flea had taken a blood meal.

These fed fleas could have been transported by pets from outdoors and then were caught in the indoor trap, or transported from other locations within the home other than the trap site. Therefore, inclusion of fed fleas may not represent newly

Figure 2. *Flea burden trends seen for total fleas on-pet and for newly emerged fleas in a household with limited external flea contamination sources, with monthly on-animal treatment of all dogs and/or cats in the household. However, in this case, existing pre-adult household burdens were high, referred to as a "Red-Line" household (20% or greater increase in flea emergence following initial treatment).*

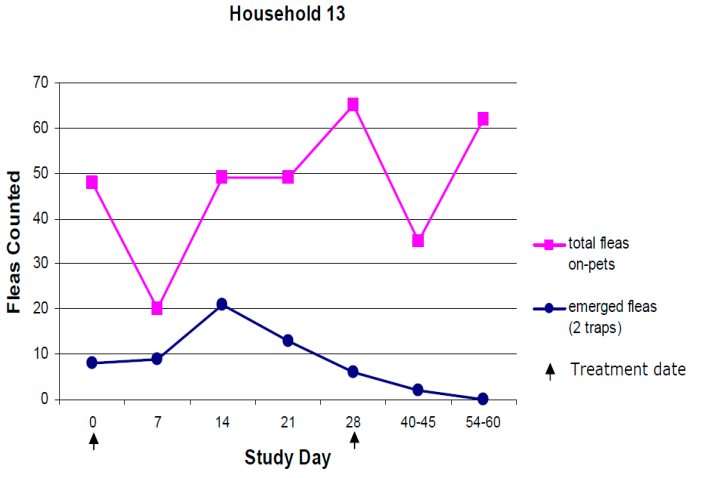
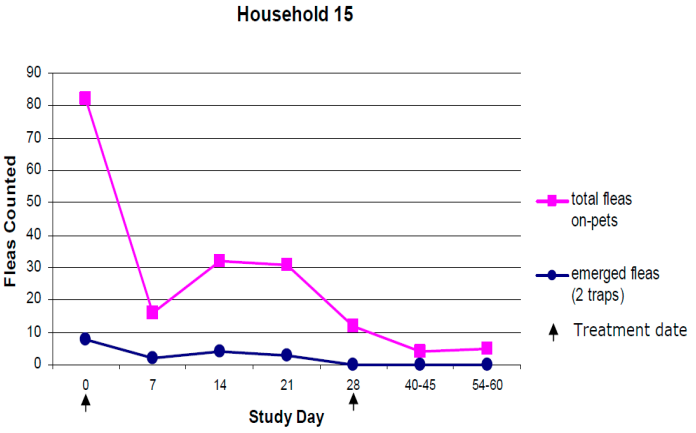


Figure 3. *Flea burden trends seen for total fleas on-pet, total fleas (fed + unfed) captured in traps, and for newly emerged fleas in a household with external flea contamination sources, and household visits by an untreated "visitor" pets. Since all household pets were treated monthly, these trends would indicate an external infestation source with access to the interior of the household. This interpretation was validated as factual, through subsequent questioning of the homeowners.*



emerged fleas from the trap location the in-home environment. Several factors likely lend to the presence of fed fleas in the traps. Pet grooming activity could be expected to dislodge fleas, which might then have been attracted to the intermittent light traps.[12, 13] Fipronil causes hyper-excitation of affected insects.[14,15] Fleas so stimulated might have fallen off the pet due to an inability maintain their grasp, or due to the pet grooming activity resulting from the atypical movement of affected fleas. Another possibility is that before establishing themselves as permanent ectoparasites, there may be a period when a small percentage of fleas display inter-host movement, which has been previously described.[16]

It has been demonstrated that the weight of newly emerged unfed female fleas increased by 51% and 91% within 12 and 24 hours respectively, after being placed on cats.[17] It was noted in the results that only nine female fleas, or 1.2% of fed fleas or 0.4% of the total number of fleas collected in the traps, appeared to be visibly engorged, indicating that most fed female fleas, if they had fed on a dog or cat, had been on that host for likely less than 12 hours. In addition, a number of these fleas might have fed briefly on humans within these households. Because male fleas feed sparingly, with minimal increase in body size and weight,[17] it was not possible to estimate the length of time fed males caught in traps might have resided on these pets.

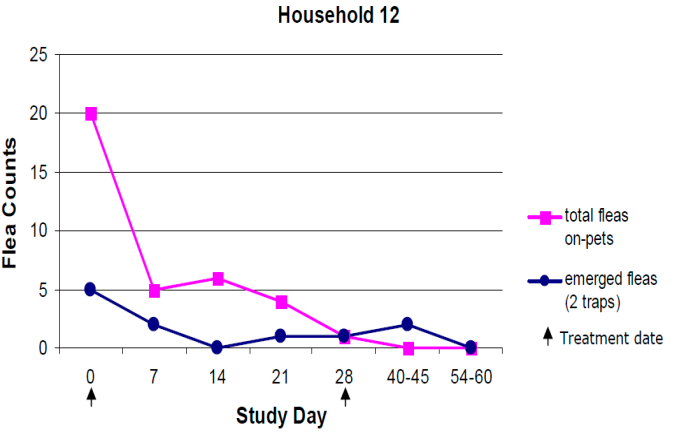
The unfed fleas afford the most accurate measurement of newly emerged fleas from the trap site in the home. Inclusion of fed or partially fed fleas would confound the area assessments of household burden and of household sex ratio, because the fed fleas could have been acquired from a number of locations other than the trap site.

The biology of Ctenocephalides felis, the cat flea is highly complex and intricately tied to environmental conditions and the availability of suitable hosts. Temperature directs the rate of development of the immature stages of the cat flea, and cool temperatures can prolong the time it takes for eggs to develop to adults.[1] Alternately, increased temperatures can rapidly accelerate development of existing pre-adult stages to emergence as young adults. Such accelerated development can lead to pet owners seeing dramatic increases in flea burdens in their homes, on their pets, and even on themselves, given that the right conditions existed in their home or outdoors. As previously discussed, it was noted that in some cases after all dogs and cats in a household were treated, the environmental flea emergence trap counts might increase within 1 to 3 weeks following treatment.[7] In these "red-line homes" ($\geq$ 20% increase), the flea population trend indicates that at the time of treatment the population was either in a rapid growth phase, or the development

and emergence of fleas at the initial assessment was delayed by environmental conditions (ie, cool ambient temperatures).

In this study, many households were enrolled during a period of unusually cool temperatures for the month of May in Tampa, FL. During the first week of the study, mean daily temperatures in Tampa ranged from 2.5 to 5^{0}C below normal (National Weather Service Data). It is likely that these lower temperatures reduced or caused a delay in the emergence of fleas at the beginning of the study and may have reduced their response to the intermittent-light flea traps.[18,19] Then as environmental temperatures warmed during the 2nd and 3rd weeks of the study, existing pre-emerged adult fleas emerged in larger numbers.

Figure 4. *Flea burden trends seen for total fleas on-pet and for newly emerged fleas in a red-line-home, where on-animal treatment of all dogs and/or cats was given monthly. The dog experienced severe exposure to fleas outdoors, documented by a site visit and observation of newly emerged fleas on the investigator. A wildlife source (raccoon) of yard contamination was acknowledged by the homeowner. High numbers of newly emerged fleas were acquired by the dog each time it went out to the back yard, but these fleas weren't able to reproduce, as newly emerged household flea populations fell to zero.*



The percentage of red-line homes seen in this study (44%) is higher than the highest percentage of red-line homes ever recorded by the author in any previous or subsequent Tampa study.[2,3,4,5,20] Importantly, due to this delayed emergence of fleas in many homes and delayed development of the pre-existing cohort of eggs, larvae, and pupae, the emergence and elimination of existing flea burdens was not completed within the typical 2-month post-treatment period normally seen in Tampa. Thus, this development delayed the level of flea clearance seen in previous in-home studies. Interestingly, one calendar year after the present study, another study using FRONLTINE Plus was performed in Tampa, FL.[20] The latter study, performed used many of the same households as this study, was performed during more typical weather patterns for Tampa. Thus, the clearance of existing infestations in FRONTLINE Plus-treated households[1] later, was more rapid and the efficacies higher by Day 60 than those seen in this study by Day 60. Even though the differing weather patterns led to different elimination rates in the two studies. In each study, as the preexisting population of fleas developed and emerged, they were eliminated following the on-animal treatment. Thus, the newly emerged fleas did not lend to development or maintenance of the household flea population in either study, and the result was an a rapid decline in household flea numbers in both.

The initial sex ratios of fleas caught in the traps in these homes is also evidence of delayed flea development and emergence in "red line" homes. The sex ratio of fleas in the traps in all homes at the beginning of the study was 1.4:1 (F:M). However, the sex ratio of fleas caught in red-line homes on day 0 was 1.9:1 (F:M). These data indicate that immature flea development in "red-line" homes was in an earlier stages of development due to the higher percentage of female fleas.

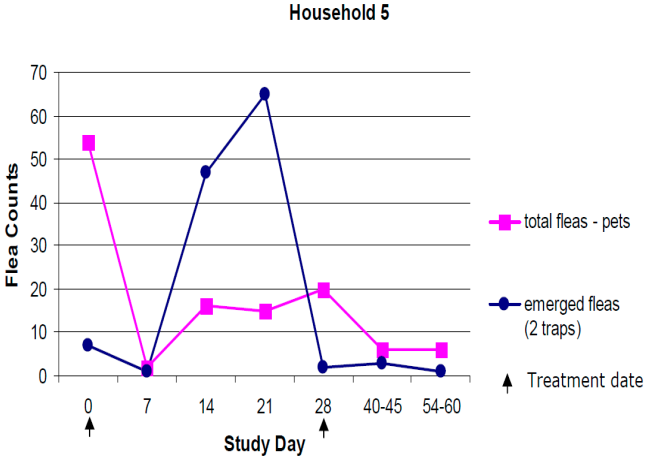
These data illustrate the temperature-dependency of fleas and the fact that slight changes can dramatically affect emergence and the length of their life cycle. In fact, a solid understanding the impact of temperature, humidity, the presence of untreated pets, feral cats and dogs, certain wildlife species, and other nuances of the flea life cycle, are critical to ensure appropriate interpretations are made, and measures are taken to ensure that effective flea control is achieved. Without consideration of these factors, it is easy for pet owners, as well as veterinary clinic staff, to assume a product is not working, rather than investigating and attending to the actual causes of continued flea presence in the face of effective treatment.

The sex ratio assessments prospectively performed in this study confirmed that the newly emerged, unfed flea populations in the homes aged following treatment of all household dogs and cats with FRONTLINE Plus. This population senescence was a result of interference with flea reproduction on treated pets. Without replenishment of flea eggs in the environment, the fleas emerging and caught in the traps were limited to those that originated from eggs laid on pets prior to treatment. As predicted, female fleas, which emerge from the existing egg cohort sooner than males, outnumbered male fleas early in the study, but by the end of the study, fleas caught in emergence traps were predominantly male.

Historically, pet flea counts have been thought to be a good indicator of household flea burdens, often such on-animal counts directly correlate with the household environmental flea burden. However, at some sites in our study, there was a great inconsistency in the on-animal findings, especially when compared to the numbers of unfed fleas trapped in the household. These inconsistencies clearly called into question the reliability of the pet flea counts in some homes, and thus precipitated more in-depth site investigations to determine the root-cause.

In some households, where both the on-animal and flea trap populations fluctuated, not only were the unfed flea numbers atypical, but microscopic examination of the trapped fleas revealed fed fleas and also the presence of engorged fleas (and in one case, an engorged and gravid flea) and the sex ratio shift transition wasn't consistent for the unfed fleas collected in the traps from those households. Site investigations and questioning of persons living in these homes revealed that the source of engorged fleas and a subsequent increase in newly emerged flea numbers observed in the house (traps) and yard (on-animal counts) were due to untreated visitor-pets in the home. In all cases, both the physical presence of the engorged female fleas found in the traps and the subsequent bloom in household flea emergence in the home, could be directly and temporally tied to specific visits of untreated animals in these homes.

Figure 5. *Flea burden trends seen for total fleas on-pet and for newly emerged fleas when on-animal treatment was given monthly, with an outside contamination source. Note that while fleas continued to be found on the animal area-counts, the household trap counts were zero by Day 28 and remained zero, again indicating complete efficacy even with a continuous challenge.*



In other study households, on-animal flea counts at the end of the study were much higher than household un-fed flea trap counts, which were low or consistently zero. The low trap counts indicated the house was not the source of these heavy on-animal flea burdens. In these cases, retrospective site investigations determined that the higher pet flea burdens seen on individual pets in the household were acquired from outdoor sources. In many of these cases, there was direct observation of a competent host, such as feral cats, opossums, or raccoons in the yard by the homeowner or the investigator. The importance of a competent outdoor source was often verified by observation of newly emerged fleas jumping onto the socks and lower legs of investigators as they moved through the suspected flea-source locations in the yard (ie, under a pool deck). In other cases, the pets themselves demonstrated the presence of outdoor flea populations. Pets just examined, combed and known to be free of fleas, were briefly allowed access to suspected outdoor flea-source areas in the yard (ie, lean-to shed, under bushes), then reexamined. Newly emerged fleas were found on the pets immediately after their brief exposure to the infested yard or environment.

In household studies, pets will typically acquire new fleas throughout the day from both indoor and outdoor sources. Therefore, in many cases, fleas counted on treated pets may have been acquired immediately prior to flea counts being conducted. This contrasts with laboratory point-efficacy studies, in which a defined number of fleas are placed on treated and non-treated animals at specified intervals, and flea counts are subsequently conducted, allowing measureable flea-product efficacy on the animals. Some pets in this field study were found to have acquired large numbers of fleas each time they went outdoors, and therefore, these pets had recently-acquired fleas on them at every assessment period throughout the study (Figure 2). However, the same household measurements continued to fall to zero or maintained at zero, even though newly acquired fleas were present on these indoor-outdoor pets. The net effect is the newly acquired fleas were not reproductively viable, either dying prior to reproducing, or the IGR activity of the product used in this study prevented adult flea development, as household infestations continued to abate.

On-animal flea counts remain an effective indicator of adulticidal flea activity within a specified timeframe in laboratory point-efficacy studies. However, in household field studies, on-animal flea assessments can be a poor indicator of both adulticidal flea activity, as well as the overall control of household infestations provided by an effective adulticide/IGR product, such as FRONTLINE Plus.

CONCLUSIONS

The data reported here demonstrate that 10 years following the introduction of fipronil/(*S*)-methoprene for topical application to dogs and cats to the US veterinary profession, FRONTLINE Plus remains highly effective for controlling flea populations, even under conditions that favored intense flea development and delayed emergence of fleas in home environments. The data also indicate that microscopic examination of flea-emergence traps provided important insights into flea population kinetics in the homes. Examination allowed assessment of the female to male ratios and fed status of fleas, which provided an accurate indication of a rising, persistent or declining flea population. Additionally, the microscopic examination of the flea traps provided important insights on the study compliance in the household. When fleas were still observed on pets or in traps at the conclusion of the 60-day trial, these observations afforded the opportunity and justification for further questioning and in-depth household investigations. In every case, investigators were able to determine the reason for the continued presence of fleas, and none were due to product failure.

The continued presence of fleas on pets and in these homes were the result of untreated visitor pets, and the presence of

feral cats, raccoons, and opossums in the homeowners yards. It is likely that most typical pet owners will have to deal with one or more of these same challenges in their homes or yards. When such challenges exist, effective flea control products should be used continuously on all pets in the household, rather than seasonally or episodically on a portion of the household pets, in order to prevent flea reproduction from occurring in the household.

ACKNOWLEDGEMENTS, FUNDING AND CONFLICT OF INTEREST STATEMENTS:

This study was funded by Merial Limited, Duluth, GA. Dr. Dryden performs consulting work for Merial Limited and other pharmaceutical companies. Dr. Carithers is an employee of Merial Limited and participated in the study design, analysis, interpretation of the data, and writing and submission of the manuscript for consideration. Dr. Gross is an independent biostatistician who performs consulting work for Merial Limited and other pharmaceutical companies. We thank Dr. J Barrie and all the staff at Sunshine Animal Hospital, Tampa FL for their invaluable assistance in conducting this study.

REFERENCES

1. Silverman J., Rust M.K., Reierson D.A., 1981. Influence of temperature and humidity on survival and development of the cat flea, Ctenocephalides felis (Siphonaptera: Pulicidae). *J. Med. Entomol.*18, 78–83.
2. Dryden M.W., Perez H., Ulitchny D., 1999. Control of flea populations on naturally infested dogs and cats and in private residences with either topical Imidacloprid spot application or the combination of oral lufenuron and pyrethrin spray. *J. Am. Vet. Med. Assoc.* 215, 36-39.
3. Dryden M., Magid-Denenberg T., Bunch S., 2000. Control of fleas on naturally infested dogs and cats and in private residences with topical spot applications of fipronil or imidacloprid. *Vet. Parasitol*. 93, 69-75.
4. Dryden M., Maggid-Denenberg T., Bunch S., Schenker R., 2001. Control of Fleas on Dogs and Cats and in Private Residences with the Combination of Oral Lufenuron and Nitenpyram. *Vet. Ther.* 2, 208-214.
4. M. Dryden, S. Burkindine, T. Lewis, L. Houdeshell, I. Rodriquez and R. Hack, Efficacy of selamectin in controlling natural flea infestations on pets and in private residences in comparison with imidacloprid and fipronil, Proceedings Am. Assoc. of Vet. Parasitol. Annual Meeting July 14–15, Boston, MA (2001), p. P34.
5. Dryden M., Broce A., 1993. Development of a flea trap for collecting newly emerged Ctenocephalides felis (Siphonaptera: Pulicidae) in homes. *J. Med. Entomol.* 30, 901-906.
6. Dryden M.W., 2009. How you and your clients can win the flea control battle. *Vet. Med*-US. Supplement. March, 17-26.
7. Dryden M, Payne P, Lowe A, Mailen S, Smith V., 2007 "Why Am I Still Seeing Fleas?" Troubleshooting Client Issues with Flea Control. *Vet. Tech.* 28:5(A), 7-9.
8. Dryden M.W., Smith V., 1994. Cat flea (Siphonaptera: Pulicidae) cocoon formation and development of naked flea pupae. *J. Med. Entomol*. 31:272-277.
9. Dryden M., Boyer J., Smith V., 1994. Techniques for estimating on animal populations of Ctenocephalides felis (Siphonaptera: Pulicidae). J. *Med. Entomol*. 31, 631-624.
10. Dryden M.W., 1989. Host association, on-host longevity and egg production of Ctenocephalides felis felis. *Vet. Parasitol*. 34, 117-122.
11. Hudson B.W., Prince F.M., 1958. A method for large scale rearing of the cat flea, Ctenocephalides felis felis (Bouché). Bull. World Health Organ. 19, 1126-1129.
12. Wade S.E., Georgi J.R., 1988. Survival and reproduction of artificially fed cat fleas, Ctenocephalides felis Bouché (Siphonaptera: Pulicidae). *J. Med. Entomol*. 25, 186-190.
13. Bloomquist J.R., 1996. Ion channels as targets for insecticides. *Annu. Rev. Entomol.* 41, 163-90.
14. Hosie A.M., Baylis H.A., Buckingham S.D., Sattele D.B., 1995. Actions of the insecticide fipronil, on dieldrin-sensitive and- resistant GABA receptors of Drosophila melanogaster. *Brit. J. Pharmacol.* 115, 909–912.
15. Rust M.K., 1994. Interhost movement of adult cat fleas (Siphonaptera: Pulicidae). *J. Med. Entomol.* 31, 486-489.
16. Dryden MW. Blood Consumption and Feeding Behavior of the Cat Flea, Ctenocephalides felis felis (Bouché 1835) 1990, Purdue University. P 12.
17. Osbrink W.L.A., Rust M.K., 1985.Cat flea (Siphonaptera: Pulicidae): factors influencing host finding behavior in the laboratory. *Ann. Entomol. Soc. Am.* 78, 29-34.
18. Silverman J., Rust M.K., 1985. Extended longevity of pre-emerged adult cat flea (Siphonaptera: Pulicidae) and factors stimulating emergence from the pupal cocoon. *Ann. Entomol. Soc. Am*. 78, 763-768.
19. Dryden MW, Payne PA, Smith V, Riggs B, Davenport J, Kobuszewski D., 2011. Efficacy of dinotefuran–pyriproxyfen, dinotefuran–pyriproxyfen–permethrin and fipronil–(S)-methoprene topical spot-on formulations to control flea populations in naturally infested pets and private residences in Tampa, FL. *Vet Parasit.* In Press, Available online 12 June 2011.