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Pilot study to assess the effects of early flea exposure on the development of flea hypersensitivity in cats

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This pilot study was to determine if early oral flea exposure reduces the incidence of flea allergy dermatitis (FAD) in cats. Eighteen kittens, assigned to three groups, received no flea exposure, oral flea exposure or flea infestation for 12 weeks. Then all the kittens were exposed continually to fleas for 31 weeks. Sensitization was monitored using intradermal testing (IDT), in vitro measurement of anti-flea saliva immunoglobulin E (IgE) and development of FAD. There was no statistically significant difference between groups in IDT reactions, in vitro data or clinical scores. The development of FAD was not associated with the presence of anti-flea saliva IgE. However, the development of a delayed reaction to flea bite was associated with symptoms after flea exposure. Although not statistically significant, the FAD scores in the oral group were lower than in the controls. Further studies are required to determine the role of oral flea exposure in the development of FAD in cats.

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Introduction

Flea allergy dermatitis (FAD) is the most common skin disease of cats and dogs worldwide (Baker 1977, Scott et al 2001). In the cat, the clinical syndromes of miliary dermatitis, symmetrical self-induced alopecia, and feline eosinophilic plaque have been attributed to flea allergy (Kunkle and Halliwell 2003). Although the recent development of new insect adulticides and development inhibitors has brought significant relief to pets, they are unlikely to eliminate the problem of FAD. Flea resistance, environmental concerns, and damage to beneficial insect populations continue to be relevant factors. The prevention of FAD would therefore be preferable to therapy after the development of the disease, but a clearer understanding of the pathogenesis of FAD is needed before vaccination or immunomodulation is likely to be successful.

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Insect hypersensitivity is recognized in most mammalian species, but considering the prevalence of insects in the environment, a biological mechanism that protects the majority of animals from developing hypersensitivity reactions must exist. Most animals in flea-rich environments can have a moderate flea population and be clinically normal while others develop FAD. Little is known regarding the specific factors that result in the development of flea hypersensitivity versus anergy, tolerance, or desensitization in the natural environment.

It is recorded that clinically normal stray dogs from pounds and asymptomatic pet dogs with continual flea exposure have low or undetectable antibodies to flea antigen (Halliwell and Longino 1985). Dogs exposed to fleas at a young age reportedly are less likely to develop hypersensitivity and resultant signs than are dogs first exposed to fleas late in life (Halliwell et al 1987). Outdoor dogs in flea-rich areas are less likely to be flea allergic than indoor/outdoor pets (Halliwell 1984). Following these observations, Halliwell and others showed that flea hypersensitivity could be induced experimentally in dogs with limited exposure to

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flea bites (Gross and Halliwell 1985, McCall et al 1998).

Little is known about the pathogenesis of FAD in the cat (Scott et al 2001). Both immediate and delayed type flea hypersensitivity occur in cats (Lewis et al 1999). Epidemiologic data regarding the incidence of FAD in cats from different environments are unavailable. Entomologists using laboratory cats for flea rearing acknowledge that these cats are at high risk of developing flea hypersensitivity (McDonald et al 1998). There is supporting evidence that cats can be sensitized to fleas with continual exposure (McCall et al 1997).

Immune tolerance is defined as a state of systemic hyporesponsiveness to an antigen, and it is considered to be of vital importance in providing for self–nonself discrimination. Immune tolerance can be initiated by antigen exposure parenterally, by inhalation or the oral route. Oral tolerance is defined as the decreased ability to stimulate a systemic immune response to antigens that were previously ingested (Faria et al 1993). Oral tolerance has been reported to block immediate reactions (anaphylaxis on challenge) as well as to decrease the occurrence of delayed type hypersensitivity (Freidman et al 1994, VanHoogstraten and Blomberg 1994). There are multiple mechanisms of oral tolerance induction, and the antigen dose, timing, and method of presentation are critical factors (Strobel & Mowat 1998).

It seems likely that oral tolerance is of major importance in the immunologic response of the cat, considering that many foreign antigens the cat encounters will be ingested through the normal grooming process. We hypothesize that oral tolerance is the mechanism that permits cats to live in flea-rich environments without sensitization and clinical reactivity. From the age at which the queen discontinues grooming her kittens and they begin to groom themselves, cats in a flea-contaminated environment are likely to regularly ingest whole fleas. We propose that through this natural exposure, most cats become tolerant to fleas and are protected from later developing hypersensitivity. Although very little work has been documented with regard to oral tolerance in the cat, it most likely occurs at a young age, and as in other forms of tolerance induction, it is related to the amount and frequency of the antigen exposure. The objective of this pilot study was to mimic the natural situation with regard to frequency and quantity of flea exposure in a flea-rich environment to determine if we could prevent the development of clinical allergy and/or flea hypersensitivity as measured by an in vitro IgE test.

Materials and methods

Facilities

This project was approved by the Institutional Animal Care and Use Committee. Cats were housed in facilities with a 12 h light/dark cycle and room temperature maintained at 75 degrees Fahrenheit (23°C). Water and food were freely available.

Animals

Queens Six specific pathogen free (SPF), flea-naive queens estimated to be 6 weeks pregnant were obtained from Liberty Research Laboratories Inc.[a] Queens were tested FIV and FeLV negative upon acquisition. Queens were housed individually in a facility free of flea exposure. At week 0 of the study, each queen was (1) intradermal tested with both Greer mixed whole body flea antigens[b] (WBF) and Heska[c] flea salivary antigens (FSA), (2) fed upon by fleas through a flea chamber and membrane, and (3) serum was collected for ELISA testing of immunoglobulin E (IgE) against flea antigens (McCall et al 1997).

Kittens Twenty kittens were born from these six queens over a 3-week period. Kittens were housed with the individual queens until they were 9–12 weeks of age. All kittens were vaccinated with feline panleukopenia/rhinotracheitis/calicivirus vaccine[d] at 6–9 and 14–16 weeks of age.

Study design

The study consisted of two phases, early and late flea exposure. Three groups of six kittens each received either no flea exposure (Group C—controls), daily oral only flea exposure (Group O), or continual flea exposure (Group F).

Phase I—early flea exposure When the kittens were 9–12 weeks of age (mean=11.14, median=11.86) the following baseline data were

[a]Liberty Research Inc., 170 Route 17 C, PO Box 107, Waverly, NY 14892, USA.
[b]Greer Laboratories, PO Box 800 Lenoir, NC 28645. USDA Category Mixed Flea Antigens from whole body *Ctenocephalides canis* and *C. felis* extracts.
[c]Heska Corporation, 1613 Prospect Pkwy, Ft Collins, CO 80525, USA.
[d]Felocell CVR®, Feline Rhinotracheitis-Calici-Panleukopenia Vaccine, Pfizer Animal Health.

gathered: (1) physical examination, (2) body weight, (3) serum collection, (4) intradermal testing (IDT) with WBF and controls, and (5) flea feeding for 5 min with flea chambers and immediate observation (15 min) data recorded.

The kittens then were allocated to one of three groups (week 0), with kittens from the same queen placed into different groups to allow for genetic variation. This pilot study used 18 kittens housed in large runs (three to six kittens/run).

Group C kittens served as controls and were housed in a flea-free environment with no flea exposure until week 16. Group O kittens had no environmental flea exposure and were housed in the same room but in different runs from Group C kittens. Group O kittens received daily oral administration of freshly thawed fleas as described subsequently. The water and fleas were squirted onto the caudal tongue and kittens allowed to swallow. Each kitten was checked immediately
to ensure that the fleas were ingested. Group F kittens were housed in a separate room within facilities similar to those of Groups C and O, but the facilities were altered to allow continual flea exposure. Ten newly emerged fleas were released onto each kitten in this group weekly and carpet squares were maintained in bedding boxes to encourage continual flea exposure via flea breeding. It was anticipated that Group F kittens would not only have fleas biting them but that they would ingest fleas through normal grooming. As the Group F kittens increased in size, the number of fleas placed weekly on each kitten was increased to 25 fleas/kitten for weeks 8–16. These kittens were flea combed weekly to confirm infestation.

Serum was collected and frozen at −70°C from kittens of all three groups at weeks 0, 5, 8, 12, and 16. Other than the testing done at week 0, serum collection was the only testing done on all kittens during Phase I.

Phase II—sensitization At the end of week 16 (kittens 26–29 weeks of age), all kittens were bled, skin tested, and again exposed to flea bite via flea chambers. Observations of IDT and flea feeding sites were made only for immediate reactivity at 15 min. All kittens were moved to the same area as Group F kittens, an area that permitted ongoing exposure and flea infestation. Kittens at this time were housed in one room with several runs and multiple cats per run. Kittens from Groups C, O, and F were mixed throughout the runs. Bedding boxes with carpeting were present in each run. Virgin fleas newly emerged from pupae were released onto all kittens. Initially 50 fleas were placed on each kitten for weeks 17 and 18, and since weekly flea combing confirmed that a population was being maintained, the number was decreased to 25 fleas per kitten weekly for weeks 19–43.

During Phase II, all 18 kittens were bled, intradermal tested, and flea chamber tested at weeks 16, 20, 24, 30, 33, 38, and 43. At week 43, all cats were clinically assessed for signs of flea allergy.

Fleas

All fleas were obtained from Heska flea insectary (Thomas 1997) as newly emerged unfed fleas. All fleas used throughout this study were unfed prior to use so they contained no foreign (non-flea) antigens.

Fleas fed to Group O kittens were prepared and frozen prior to their use in Phase I. Newly emerged unfed flea bodies were lightly crushed with a mortar and pestle to damage the chitinous exoskeleton and mimic natural trauma as is likely to be inflicted on fleas by cats during normal grooming. Fleas were frozen in 0.5 ml aliquots of water, three fleas/aliquot, and thawed daily prior to oral administration.

Intradermal testing

The IDT included WBF at 1:1000 w/v dilution as well as a negative control (Greer allergen diluent of phosphate buffered saline and phenol) and positive control (histamine phosphate 1:100,000 w/v). All kittens were skin tested at weeks 0, 16, 20, 24, 30, 33, 38, and 43 with WBF. Kittens were tested with FSA at a concentration of 1 µg/50 µl per injection site on only two occasions (weeks 16 and 43 and the end of Phase I and Phase II). The FSA was prepared from saliva collected from newly emerged unfed fleas, as previously described (Frank et al 1998).

A volume of 0.05 ml of each substance was injected intradermally on the clipped lateral thorax. All injection sites were examined for erythema, induration and size of the wheal, and a subjective score assigned from 0 to 4+, as compared with the negative and positive controls. An immediate reaction given a 2+ or greater score was considered positive. Intradermal test sites also were examined visually at 48 h for delayed type reactions at the beginning (week 0) and the end of the study (week 43).

If necessary, the kittens were sedated with ketamine hydrochloride 5–10 mg IV for IDT. Since

Table 1. Results of all tests for FAD before and after 31 weeks of fleas in kittens treated with oral (Group O), continual (Group F), or no (Group C) prior flea exposure

Cat ID	Before (week 0)				After (week 43)						
	Clinical score	IDT	Elisa (OD)		Clinical score[a]	IDT		Elisa (OD)[b]		Flea feed site[c] immediate[d]	IDT[e] DTH[d]
		WBA	WBA	FSA		WBA	FSA	WBA	FSA		
C-1	0	0	0	1	11	4	4	**210**	**3693**	+	–
C-2	0	0	8	0	0	3	4	47	**630**	+	–
C-3	0	0	0	10	16	0	4	5	**418**	+	+
C-4	0	0	0	5	0	4	4	**162**	**2714**	+	–
C-5	0	0	0	1	25	0	1	9	73	+	+
C-6	0	0	0	4	0	0	0	33	**2276**	+	+
F-1	0	0	0	3	7	0	4	95	**3124**	+	+
F-2	0	0	0	4	15	4	2	**1357**	**141**	+	–
F-3	0	0	0	4	3	0	0	32	**660**	+	+
F-4	0	0	0	4	0	1	0	2	10	–	–
F-5	0	0	0	6	10	2	2	**264**	**2977**	+	+
F-6	0	0	0	5	1	0	4	**1186**	**3579**	+	+
0-1	0	0	0	1	0	0	4	16	**1415**	+	–
0-2	0	0	0	5	0	0	2	14	58	+	–
0-3	0	0	0	4	5	0	0	28	**339**	+	–
0-4	0	0	0	3	0	0	2	5	**161**	–	–
0-5	0	0	0	3	0	0	0	**168**	**3404**	+	–
0-6	0	0	0	0	3	4	4	**441**	**3606**	+	+

FAD, flea allergic dermatitis; IDT, intradermal test; OD, optical density; WBA, Greer mixed whole body flea antigens; FSA, Heska flea salivary antigens; DTH, delayed type reactions.
[a] Mean scores: Group C=8.7, Group F=6.0, Group O=1.3.
[b] Values reported in bold were considered positive (>200 OD).
[c] With Heska fleas in chambers.
[d] Reactions were negative in all kittens at week 0.
[e] With Greer flea antigen.

this IDT was so minimal (three to four injections), it often was not necessary.

Flea chamber feeding

Kittens were subjected to flea feeding via the use of a small flea 'chamber'. The chamber was constructed from a plastic test tube into which 8–10 newly emerged adult fleas were placed and then a thin mesh (nylon stocking) was stretched over the end of the tube and held in place with a rubber band. The chamber was applied to the clipped skin of the cat and held in place for 5 min. Fleas were noted to begin feeding immediately. This site was monitored visually for 15 min for the development of a wheal and flare reaction at feeding sites. New fleas were used for each cat. A reaction to flea feeding was considered positive if there was evidence of small wheals or papule(s).

ELISA for IgE antibody to FSA

This procedure has been described elsewhere (Stedman et al 2001). Values of >100 optical density (OD) were considered positive.

Clinical evaluation of cats

One investigator independently evaluated all cats for signs of FAD at weeks 0 and 43. A previously validated clinical assessment scoring system for FAD signs was used (Kunkle et al 2000).

Statistical analysis

Prior to the start of the study, a power analysis was performed using group means and variance that would be expected in each group. With an alpha of 0.05 and a power of 0.95, a sample size of six animals/group was found to be most likely to detect a statistically significant difference between groups.

Data from the ELISA assay were analyzed using least squared analysis of variance (LSANOVA) of a split plot design (Phases I and II) with all main effects and appropriate interactions included in the model. Both Kruskal–Wallis and ordinary ANOVA were used to evaluate clinical signs scores among cats and within each group.

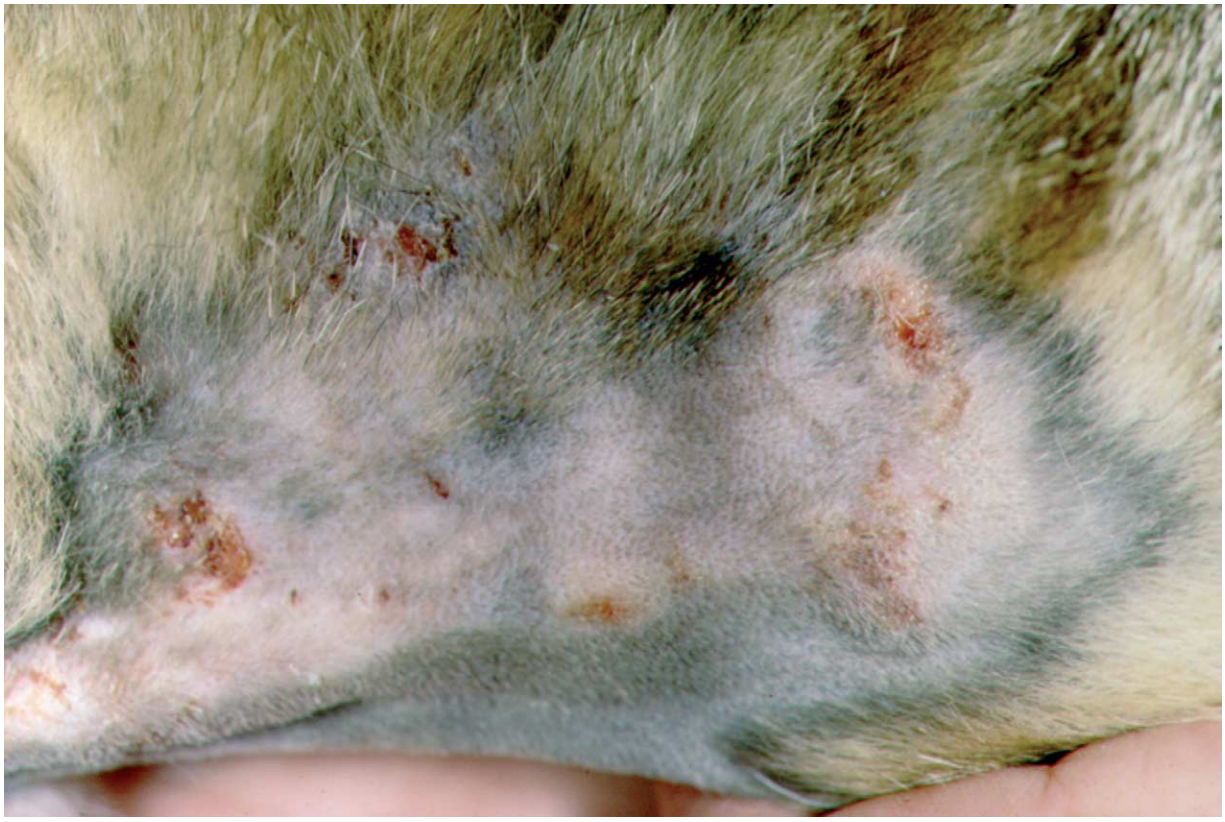

Fig 1. Clinical signs of military dermatitis, which were fairly generalized, in control cat number 5 (C5). This cat had the highest clinical score and was positive at week 43 both to IDT and delayed type hypersensitivity to flea feeding.

All statistics were performed using SAS Statistical Software, version 8.2.[e] A $p \leq 0.05$ was considered significant.

Results

Assessment of queens

All queens were negative on IDT, flea chamber feeding, and ELISA for WBF and FSA reactivity at week 0. These results confirmed that these queens had not previously been sensitized or likely exposed to fleas.

Clinical evaluation of kittens (Table 1)

At the beginning of the study (week 0), all kittens were clinically normal with clinical score indices of 0. Three of six control kittens, 5/6 early flea exposure kittens, and only 2/6 orally exposed kittens had signs of FAD at week 43. However, the differences were not statistically significant. See clinical FAD in C5 kitten (Fig 1).

[e]SAS Institute, Cary, NC.

Intradermal testing (Table 1)

All kittens at week 0 and week 16, including those with continual flea exposure, exhibited negative IDT reactivity to WBF. Kittens from all three groups also were tested with FSA at week 16. Three kittens, one in each group, had positive immediate intradermal FSA reactions. One cat in Group F had a 4+ reaction to FSA, while two other cats had 3+ (Group O) and 2+ (Group C) reactions (data not shown).

By the end of the study (week 43), six kittens exhibited positive immediate IDT reactivity to WBF and 12 kittens were IDT positive to FSA. There was no statistically significant difference in immediate skin test reactivity to WBF or FSA between the groups.

ELISA testing (Table 1)

Only 4/6 kittens in Group F showed detectable serum IgE in Phase I of the study (data not shown). Once all kittens were exposed to fleas, in addition to being subjected to repeated IDT with flea allergens, most developed measurable serum

IgE specific for FSA. By the end of the study, 5/6 kittens in each group had measurable serum anti-FSA IgE. Group F had significantly higher anti-FSA IgE than Groups C and O at weeks 16, 20, and 22 (data not shown). Fewer kittens had IgE to the WBF, although one kitten (Group F) developed IgE to this allergen preparation earlier than to the FSA. The ELISA values using WBF were generally lower, and more variable over time. By the end of the study, 7/18 kittens had serum IgE to this allergen. There were no differences between groups F, C, and O over all times. Phase II WBF and FSA ELISA values were significantly greater than Phase I values ($p=0.0198$).

Flea chamber feeding (Table 1)

All kittens were flea chamber fed at week 0 and had no reactivity at the site of flea feeding. At week 16, all kittens including those in Group F, who had continual flea exposure, were still negative to flea bites through feeding chambers when observed at 15 min (data not shown). Only at week 20 did some kittens develop papules at the site of flea feeding (data not shown). At the end of the trial, 16 kittens showed immediate papules or wheals after flea feeding. Eight kittens had palpable or visible delayed reactions at 48 h to the flea feeding site. There was no statistically significant difference between the groups.

Discussion

The purpose of this pilot study was to test two hypotheses: (1) early exposure of kittens to fleas can prevent the later development of flea sensitization and (2) the route of exposure is important. After various methods of early exposure, kittens were sensitized to fleas by continual exposure and the induction of flea hypersensitivity was assessed by clinical signs, examination of immediate sites after flea feeding with chambers, IDT with flea antigens, and in vitro assessment of IgE antibodies to flea antigens.

Historically, there is very little information available concerning the immunologic response of the neonatal cat. An early report (Miller-Ben Shaul 1965) showed oral tolerance could be induced in newborn and perinatal kittens. Various antigens were given orally at a dose of 1 g of various proteins via a nursing bottle for 7 days beginning on the first postnatal day. At 10 weeks of age, the kittens were challenged intravenously with antigen. It was concluded that tolerance was induced in 16 of 19 kittens. Subsequently, a group of 30 kittens was used to show that the majority of kittens would absorb oral antigens and develop immunologic tolerance up to 20 days, and rarely up to 28 days of age. From these small studies it would appear that 25–30 days of age is the maximum period of tolerance induction in cats, although a study in dogs has shown that tolerance to ovalbumin may be induced starting at 13 weeks of age (Deplazes et al 1995).

Since so little is known about the timing, the quantity, the duration, method of presentation, and other factors involved in the induction of oral tolerance in the cat, this study tried to mimic what likely would happen in nature. Thus, the kittens remained with the queens and the queens groomed them in a normal manner. The initial design of this study was to have all kittens within a few days of each other in age and to start Phase I of the study when the kittens were as close to 6 weeks of age as possible. This would mimic the more common situation in which kittens are weaned and separated from their mothers and placed into homes. Several circumstances prevented this. The first was that although all queens were purchased with similar estimated parturition dates, five queens gave birth within the same week, while one queen gave birth 21 days after the first. This meant that there were 21 days difference in the ages of the kittens. Also, an outbreak of infectious disease occurred elsewhere in the facility, not permitting us to place kittens into safe, separate facilities where we could have flea infestations. The separation of the kittens from the queens and the early exposure to fleas was delayed until the kittens were 9–12 weeks of age. This age for initial exposure to fleas, either through infestation or orally may have been late based on the little known about oral tolerance in the cat. We cannot say for certain that this was too late, however, as flea exposure in temperate climates may occur initially at this age or even later.

Other factors may have influenced the outcome of this pilot study. The amount of antigen given orally may have been insufficient. Low doses of antigen in oral tolerance induction have been shown to favor active suppression, which occurs by antigen induction of regulatory T lymphocytes that secrete immunosuppressive cytokines. Feeding higher antigen doses favors anergy and clonal deletion (Garside and Mowat 1997).

In Phase I of this pilot study it was presumed that the kittens continually infested with fleas were likely ingesting fleas as their grooming skills developed. However, they were monitored with

regular inspections and flea combing; fleas were added weekly and infestations were maintained with carpeted bedding boxes.

The kittens that received the dead fleas orally had no other flea exposure. Although this does not mimic a natural setting, the attempt was made to see if tolerance could be induced with only oral exposure to the average number of fleas estimated to be ingested with a moderate infestation. Gastric gavage was not used in the orally exposed group as it bypasses the pharyngeal lymphatics, which may be important in the induction of oral tolerance.

In Phase II of the study, sensitization to fleas was attempted with continual exposure based on previous experiences of investigators. A most recent report with 10 cats looked at the role of intermittent vs. continual flea exposure in the development of clinical signs of FAD. Colombini et al (2001) concluded that there was no difference between the groups. The longest time period for clinical signs of sensitization was 150 days. In the current study, cats had continual flea exposure for 189 days, which from all prior reports should have been adequate for sensitization in susceptible animals.

Kittens were not tested with the FSA at week 0 or at multiple points throughout the study because of the concern that it could sensitize them after only one exposure. For this reason, this antigen was used for IDT only at the end of Phase I and Phase II. This was a greater concern with FSA than WBF because FSA is a more concentrated source of antigen. The presence of a positive immediate intradermal test did not correlate with the development of symptoms, nor did a positive ELISA test for FSA or WBF predict or correlate with FAD in these cats. However, 7/10 symptomatic cats had a delayed reaction to flea bite, while only 1/8 asymptomatic cats did so. The development of the symptoms of FAD in these kittens therefore appears to be more closely associated with a cell-mediated reaction to flea bite than with an anti-flea IgE response.

Colombini et al (2001) have reported concurrent indolent lip ulcers in association with the induction of flea allergy in flea-naive cats. The appearance of indolent ulcers in cats from certain purpose bred laboratories has been documented previously (Power and Ihrke 1995). In the current study, indolent ulcers occurred in some kittens from each group.

Using this FAD scoring system, a symptomatic cat in a humane study, would never reach the maximum score, which would require severe alopecia, scaling, crusts and excoriations, erythema and papules over 100% of the body. However, even if the clinical assessments were divided into mild, moderate, and severe, overall, most of these kittens did not get marked clinical signs of FAD. This fact is interesting, and may suggest that it is more difficult to sensitize younger kittens to fleas so that they develop clinical signs of FAD. It may be that older cats, more weeks of exposure, or a larger numbers of fleas would have induced more severe clinical signs.

The cats used in this pilot study were not selected for a predisposition to atopic disease, and were somewhat heterogeneous in terms of symptom severity. Despite this observed wide range of symptoms, the cats exposed to fleas orally tended to have limited symptoms upon flea infestation, though this was not statistically different from the controls. One of the two symptomatic Group O cats had very mild but widely distributed scaling, which would have been very unlikely to cause an owner concern.

The number of cats used in this pilot study was based on a power analysis performed prior to the start of the study. Since there have been no oral tolerance studies of this type in cats, the group means and variance were estimated. Also, there are no power analyses available for a study using a repeated measures design (multiple samples taken over time from the same animals), so it is difficult to rely too much on a power analysis. The results of this pilot study provide information for a more accurate power analysis used prior to future studies investigating oral tolerance in cats.

A follow-up study using larger numbers of animals in each group should be performed to determine whether this observed trend is repeatable and clinically significant. In addition, studies varying the dose of oral flea allergens, and the age at which oral exposure is begun, are needed to define the role of tolerance in preventing the induction of FAD in domestic cats.

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