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IDENTIFICATION OF FABRICS LIKELY TO COLLECT AND DISPERSE FEL D 1

by
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B.S. Florida Southern College, 2002

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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in the College of Sciences
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ABSTRACT

Individuals sensitive to domestic cat allergen Fel d 1 experience a variety of symptoms including eye irritation, respiratory irritation, asthma, and severe respiratory distress. Fel d 1 is a protein produced in the saliva and on the skin of domestic cats. Previous studies have demonstrated that Fel d 1 adheres to clothing, upholstery, and human hair and has been found in non-cat environments in levels high enough to cause allergic reactions in sensitive individuals. In a general sense, two very different approaches have been adopted to study Fel d 1. One area of the literature focuses on the molecular biology of Fel d 1 and its functions at the cellular level. These studies hold long-term promise for an effective clinical response to this persistent allergen. An entirely separate literature focuses on immediate practical solutions that remove Fel d 1 from the domestic environment. Within this literature there has been minimal emphasis on the possibility that different fabrics may have different affinities for Fel d 1. Therefore, the affinity of Fel d 1 for different fabrics is the focus of this study. The findings from this study will be of use in reducing allergic reactions in sensitive individuals through the choice of appropriate fabrics in clothing and upholstery.

Forty domestic household cats were chosen for this study. Each cat was rubbed, in a manner similar to petting, with an assembled fabric square based on a Latin-square design. Each Latin-square design consisted of a 6x6 fabric grid and included the fabrics silk dupioni, wool suiting, cotton denim, cotton damask, polyester suede and polyester knit. The random organization of the fabrics into the grid removed bias for the location of fabrics within the square during Fel d 1 collection. After rubbing, the Latin-square fabric

block was disassembled and Fel d 1 was extracted from each fabric type and analyzed via quantitative ELISA. The results were statistically analyzed with a univariate ANOVA. Fabrics significantly differ ($p<0.001$) in Fel d 1 retention and fall into three groups. Silk dupioni collected the least amount of Fel d 1. Wool suiting, cotton denim and cotton damask were intermediate in Fel d 1 collection, while polyester suede and polyester knit collected the highest amounts of Fel d 1.

Samples were also collected for a time study to determine if Fel d 1 bound on fabric degrades, or otherwise diminishes, over time. 14 weeks (approximately 3 months) after collection, Fel d 1 was extracted from fabrics and quantified by ELISA. A paired T-test was used to evaluate changes in Fel d 1 levels on specific fabrics over the 14 week period. When compared to extractions performed immediately after exposure, the amount of Fel d 1 released from specific fabrics after 14 weeks was significantly reduced.

From these studies I conclude that an individual allergic to Fel d 1 may be able to limit their allergen exposure by selecting fabrics less likely to collect the allergen for their environment. Natural fibers (silk, wool, and cotton) collected less Fel d 1 than polyester fabrics, suggesting that natural fibers are recommended over fabrics containing polyester for persons allergic to cats.

I dedicate my thesis to my husband, Curtis Jones, who never complained during the long process to perform this research and write this manuscript and who also gave me tremendous support and encouragement during every step along the way.

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INTRODUCTION

Indoor allergens affect millions of Americans every day. Common producers of allergens are dust mites, cockroaches, cats and dogs (Hamilton 2005; Platts-Mills et al. 2007). Insect-produced allergens include Der p 1, which is produced by dust mites, and Bla g I, and Bla g II which are produced by cockroaches (Hamilton et al. 1992). Other sources of indoor allergens include cigarette smoke, mold, mice, and fungi (Hamilton et al. 1992).

Indoor pets also contribute to allergies. The most common pet produced allergen is Fel d 1, which is produced only by domestic cats (*Felis domesticus*) (Hamilton et al. 1992). Cats produce Fel d 1 in the salivary and sebaceous glands (Bartholome et al. 1985; Dabrowski et al. 1990; Mata et al. 1992). When measured on the skin, Fel d 1 can be found in the highest concentration on the face of the animal (Caroyol et al. 2000). However, when measured only on the fur, Fel d 1 is present in the highest concentrations on the neck (Avner et al. 1997).

Intact (non-neutered) males produce Fel d 1 at higher levels than females (Jalil-Colome et al. 1996), and Fel d 1 levels correlate positively with testosterone levels (Zielonka et al. 1994). Neutered males produce Fel d 1 levels similar to females (Ramadour et al. 1998). However, intact males, neutered males, and females all produce Fel d 1 at levels high enough to cause allergic or asthmatic response in sensitive individuals (Miller et al. 1995), and the presence of cats in the home has been linked to higher rates of asthma (Plaschke et al. 1999; Caroyol et al. 2000). Neutered male cats carry an average of 67 mg of Fel d 1 (range of 3 – 142 mg) on their body at any time (Avner et al. 1997). A mean dosage of 7.74 μg/ ml has been demonstrated to cause a

cutaneous reaction in Fel d 1 sensitive individuals (Kleine-Tebbe et al. 1993). Thus, it is clear that all cats have the potential to cause allergic and asthmatic symptoms in sensitive individuals.

Structure of Fel d 1

Fel d 1 is a tetrameric glycoprotein with a molecular weight (MW) of ~36KD (Leitermann et al. 1984; Duffort et al. 1991; Kaiser et al. 2007). The tetramer is composed of two heterodimers that are non-covalently linked and the two subunits are about 18KD each (Leitermann et al. 1984; Duffort et al. 1991; Morgenstern et al. 1991). The 18KD subunits each consist of 4KD α-chain and a 14KD β-chain that are linked by three disulfide bonds (Duffort et al. 1991; Kroll Kristensen et al. 1997). In addition, each subunit contains IgE epitopes, two of which are on the α-chain and a third on the β-chain (van Milligen et al. 1994; Kaiser et al. 2003). Each chain is encoded from a different gene (Griffith et al. 1992). Carbohydrates make up 20% of the Fel d 1 protein and may act to preserve the active conformation of the protein (Duffort et al. 1991).

The α-chain contains 70 amino acids and has a common polymorphism at codon 29 that replaces Asn with Lys (Morgenstern et al. 1991; Griffith et al. 1992; Kroll Kristensen et al. 1997). The frequency and effect of this polymorphism on the allergenicity of Fel d 1 in the general cat population is not known (Griffith et al. 1992). On the genomic level, the α-chain has two leader sequences termed leader A and leader B (Griffith et al. 1992). The leader A sequence is present in both the salivary glands and skin of cats, whereas the leader B sequence appears to be expressed only in the salivary gland (Griffith et al. 1992).

The β-chain is glycosylated and contains an N-linked oligosaccharide (Duffort et

al. 1991; Morgenstern et al. 1991; Kroll Kristensen et al. 1997). There are two abundant forms of the β-chain, a Long Chain (LC) and Short Chain (SC). The LC variant consists of 92 amino acids and is found primarily in the salivary glands, whereas the SC variant consists of 90 amino acids and is found primarily on the skin (Griffith et al. 1992). It is not known if the LC and SC forms are different alleles of one gene or alternatively spliced variants of a common mRNA precursor. While polymorphic variants of LC and SC have been identified, their frequency and biological role in the general cat population is unknown (Griffith et al. 1992; Kroll Kristensen et al. 1997). The β-chain also has been demonstrated to contain two T-cell epitopes that may be the sites of initial allergen sensitization in the immune system (Bateman et al. 2008).

Fel d 1 is a relatively stable protein. However, while heating of purified Fel d 1 protein diminishes but does not eliminate its allergenic properties, a marked reduction in ability to bind antibody occurs following reduction to two separate chains. This observation suggests that the IgE binding activity is dependent on conformation (Duffort et al. 1991)

Other indoor allergens, such as the common dust mite allergen Der p 1 and another cat allergen, Fel d 3, are cysteine proteases that have been demonstrated to degrade epithelial barriers that must be crossed for an allergen to cause an allergic reaction in sensitive individuals (Wan et al. 1999; Wan et al. 2000; Ichikawa et al. 2001). Fel d 1 has been demonstrated to be associated with the degradation of gelatin and fibronectin (Ring 2000), however Fel d 1 itself has not been demonstrated to be an enzyme. Fel d 1 may act in conjunction with Fel d 3, Der p 1, and other allergens to gain access to mast cells to trigger an allergic response in sensitive individuals.

The physiological role of Fel d 1 in cats is unknown. The Fel d 1 α-chain shares 54% sequence similarity with a rabbit uteroglobin precursor protein and has a similar crystal structure (Morgenstern et al. 1991; Kaiser et al. 2007). The uteroglobin protein in rabbits has been proposed to protect the wet epithelia of embryos against the maternal immune response during implantation (Morgenstern et al. 1991). Thus, it is possible that cats use Fel d 1 to protect their dry epithelia.

Immune Response

Individuals who exhibit allergic or asthmatic reactions to cat dander are sensitive to Fel d 1 (Bierman et al. 1996) and Fel d 1 elicits an IgE immune response in these individuals (Ohman et al. 1977; Lowenstein et al. 1985). The major symptom of Fel d 1 exposure is respiratory distress, such as wheezing, rhinitis, and breathing difficulty. Even small doses of Fel d 1 are associated with airway inflammatory response in sensitive individuals (Sulakvelidze et al. 1998). Using skin prick tests (SPT), a mean dosage of 7.74 μg/ ml Fel d 1 was sufficient for a positive reaction in individuals previously determined to be sensitive to Fel d 1 (Kleine-Tebbe et al. 1993). Sensitization to Fel d 1 has also been demonstrated in individuals who have no direct contact with cats (Chan-Yang et al. 1999; Liccardi et al. 2005), suggesting exposure via intermediate sources (e.g., fabrics that bind Fel d 1).

While the heterodimer has three IgE epitopes, two studies have suggested that the α-chain epitopes have greater ability to induce an IgE response in sensitive individuals. When administering an intradermal dose of 80 μg of Fel d 1 α-chain peptides, 25% of asthmatic individuals sensitive to Fel d 1 exhibited bronchoconstriction (Smith et al. 2004). In another study chemically synthesized portions of the alpha and beta chains were

reacted with the sera of patients sensitive to cat allergens. Of the 65% of tested individuals that showed IgE binding to one of the Fel d 1 epitopes, 74% reacted to the epitopes on the α-chain and only 11% reacted to the epitope on the β-chain (van Milligen et al. 1994). This suggests that even a fragment of the Fel d 1 molecule is sufficient to induce an allergic response.

Retention of Fel d 1 on Fabrics

Living with cats in the home has been shown to aggravate asthmatic symptoms in sensitive individuals, and avoidance of cats has been recommended for those individuals (Plaschke et al. 1999; Platts-Mills et al. 2007). While avoiding areas with cats can be beneficial to individuals suffering from cat allergies, Fel d 1 is ubiquitous in indoor environments such as schools, work areas, and cars where a cat has not been present (Patchett et al. 1997; Neal et al. 2002; Karlsson et al. 2004). Several studies have demonstrated that Fel d 1 is carried into cat-free environments on clothing and human hair (D'amato et al. 1997; Patchett et al. 1997; Liccardi et al. 1998; Liccardi et al. 2002; Neal et al. 2002; Karlsson et al. 2004; Karlsson et al. 2005). Thus, indirect exposure may contribute to increased asthma and allergic sensitization in sensitive individuals (Ritz et al. 2002). Given that fabrics may carry Fel d 1, it becomes important for those with cat allergies to determine which fabrics are more likely to carry Fel d 1. Wool is reported to carry higher levels of Fel d 1 relative to other fabrics; however these observations may reflect longer Fel d 1 collection intervals because wool fabrics are washed less frequently (Patchett et al. 1997). Frequently cleaned garments have lower Fel d 1 levels than items that are infrequently cleaned (De Lucca et al. 2000). Fel d 1 is a 'sticky' molecule, and even vacuum cleaning will not completely remove the allergen from fabric (Liccardi et

al. 2007). However, washing cotton fabrics with water has been demonstrated to completely remove Fel d 1 (Liccardi et al. 1998). Dry cleaning of fabrics reduces Fel d 1 levels, but with lesser efficiency than water. In addition, the dry cleaning process can contaminate fabrics previously not exposed to Fel d 1 (Liccardi et al. 2002).

Goals of this Study

Studies of the molecular structure and biological activities of Fel d 1 may lead to clinical intervention in the future. However, in the near term, investigations into the practical limitation of Fel d 1 exposure are of more value to Fel d 1 sensitive individuals. To date, no conclusive study has compared fabrics for the ability to transport the allergen. Individuals sensitive to Fel d 1 can benefit from identifying common fabrics used in clothing and home furnishings that are likely to collect and disperse Fel d 1. In addition, understanding which fabrics are more likely to retain Fel d 1 over time will aid in selection of upholstery that cannot be frequently washed.

This research addresses these concerns by quantifying Fel d 1 bound to different fabrics exposed to the protein. Initial binding and retention of Fel d 1 on fabrics are examined. The results provide a framework for fabric choice recommendations for Fel d 1 sensitive individuals.

METHODS

Fabrics Tested

Six fabric types representing a variety of fabric material and weaves used commonly in apparel and home furnishings were purchased from a local fabric retailer. Based on the findings of Patchett et al (1997), which noted that wool fabrics might be good reservoirs of Fel d 1, a wool fabric sample was included in this study. The other fabrics tested included polyester knit, polyester suede, cotton denim, cotton damask, and silk dupioni. The polyester knit, cotton denim and wool are commonly used in apparel fabrics, while the polyester suede, cotton damask and silk dupioni are common upholstery and household fabrics. All fabrics were composed of 100% of the indicated materials. No blended fabrics were used and a total of 1.3 meters of each fabric selection were purchased. After purchasing, fabrics were immediately placed in individually sealed plastic bags and stored in a cat-free environment until Fel d 1 collection. Samples of each fabric were tested for contamination with Fel d 1 prior to Fel d 1 collection experiments: no Fel d 1 was detected on the fabrics.

Selection of Cats

Forty cats were selected for study (Table 1). All cats were in good health, able to tolerate the procedure, and had no known factors, such as illness, that would preclude them from the study.

Fel d 1 ELISA

A commercially available ELISA antibody kit (Indoor Biotechnologies) was used to quantify Fel d 1 collected on fabrics. This kit has been widely used in published Fel d 1 studies (Patchett et al. 1997; Ramadour et al. 1998; Caroyol et al. 2000; Tovey et al.

2001; Karlsson et al. 2004; Karlsson et al. 2005).

ELISAs were performed according to the manufacturer's instructions, with the exception that the assay was developed with tetramethylbenzidine (TMB) instead of ABTS, an acceptable substitution allowed by the kit manufacturer. Plates were read by spectrophotometer at 405nm.

To quantify Fel d 1 collections, a standard curve was performed as part of each ELISA plate using a Universal Allergen mixture (supplied with kit). The standard curve was performed in duplicate from 100ng/mL in doubling dilutions to 0.2ng/mL Fel d 1. The unknown amounts of Fel d 1 extracted from fabrics were fitted to the standard curve using a linear regression that described the standard curve well (R^2 range = 0.97-0.99).

Table 1: Attributes of Cats used in Study

No	Age (years) at time of collection	Sex	Spayed or Neutered*	Breed**	Coat Length	Environment	Use Flea Meds
1	5	M	Y	DSH	Short	Indoor	No
2	5	F	Y	DSH	Short	Indoor	No
3	5	M	Y	DSH	Short	Indoor	No
4	11	F	Y	DSH	Short	Outdoor	Yes
5	14	M	Y	DSH	Short	Mostly Indoor	Yes
6	11	F	Y	DLH	Short/Medium	Mostly Outdoor	No
7	8	M	Y	DLH	Medium	Mostly Outdoor	No
8	<10	M	Y	DSH	Short	Outdoor	Yes
9	10	F	Y	DSH	Short	Outdoor	Yes
10	7	F	Y	DSH	Short	Indoor	No
11	4.5	M	Y	DLH	Long	Indoor	Yes
12	<1	M	Y	DSH/ Siamese	Short	Indoor	Yes
13	4.5	M	Y	DLH	Medium	Indoor	Yes
14	10	F	Y	DSH	Short	Indoor	No
15	4	F	Y	DSH	Short	Indoor	No
16	3.5	M	Y	DSH	Short	Indoor	No
17	4	F	Y	DSH	Short	Indoor	No
18	7.5	M	Y	DSH	Short	Indoor	No
19	< 2	F	Y	Abyssinian	Short	Indoor	No
20	1	F	Y	DSH	Short	Indoor	No
21	1	M	Y	DSH	Short	Indoor	No
22	2.5	F	Y	DSH	Short	Indoor	Yes
23	3	M	Y	DLH	Medium	Indoor	No
24	5	F	Y	DSH	Short	Indoor	No
25	3	F	Y	DSH	Short	Indoor	No
26	9	F	Y	DSH	Short	Indoor	No
27	6	M	N	DSH	Short	Indoor	No
28	9	F	Y	Manx	Short	Indoor	No
29	10	F	Y	Siamese	Short	Indoor	No
30	8	F	Y	Ragamuffin	Long	Indoor	No
31	4	M	Y	DSH	Short	Mostly Outdoor	No
32	4	M	Y	DSH	Short	Mostly Outdoor	No
33	8.5	F	Y	DSH	Short	Indoor	Yes
34	9	M	Y	Maincoon	Medium	Indoor	Yes
35	4	F	Y	DSH	Short	Indoor	Yes
36	7	M	Y	DSH	Short	Indoor	Yes
37	11	M	Y	DSH	Short	Indoor	No
38	5	M	Y	DSH	Short	Indoor	No
39	14	M	Y	Siamese	Short	Indoor	No
40	2.5	F	Y	DSH	Short	Indoor	No

*Spayed =20, Neutered = 19, Intact Male = 1

**DSH = domestic short hair cats, which are cats with short coats and no discernable pedigree. DLH = domestic long hair cats, which are cats with medium to long coats and no discernable pedigree.

Preparation of Latin Squares

Fabric squares were assembled with staples into a Latin square design. In the Latin square design, each fabric appears once in each of six rows and columns for a total of 36 squares (Figure 1). This was done to remove location bias of the fabrics in the quilt-square. The Latin-square design was not the experiment, but a way to collect Fel d 1 evenly on all fabric types. Random patterns were created for each fabric rectangle using an Excel macro. Staples efficiently connected fabric squares together eliminating the need for adhesives that may have interfered with the collection or ELISA assay.

Each assembled Latin Square fabric rectangle was placed in a sealed plastic bag until Fel d 1 collection (usually within 24 hours). One fabric rectangle was assembled for each cat tested. When creating the fabric rectangles, a portion of each fabric type used was reserved in a sealed plastic bag in a cat free environment to serve as a background control to be processed alongside the fabric components after Fel d 1 collection.

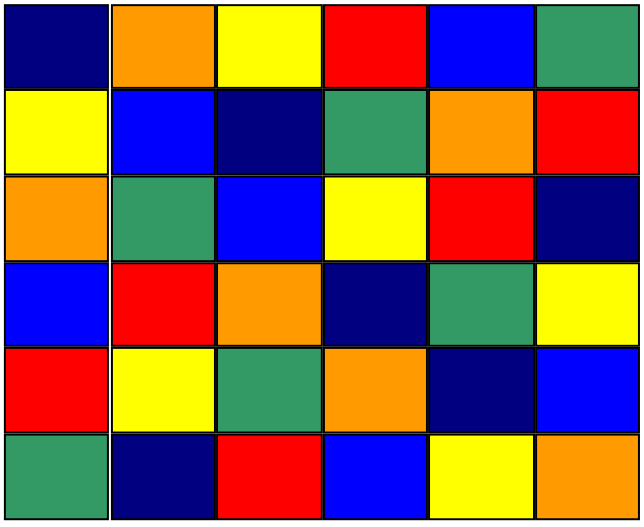

Figure 1: Example of Latin Square Based Design Block
Each fabric appears once in each row and once in each column.

Fel d 1 Collection

One fabric rectangle was rubbed on one cat (primarily on the head and neck) in a manner similar to petting for several minutes. The Fel d 1 produced around the face and neck of the animals has been demonstrated to react strongly with the monoclonal antibodies supplied in the ELISA kit (Bienboire-Frosini et al. 2009). Fabric rectangles were stored horizontally in individual zip top bags and transported to the laboratory, where individual fabric pieces were separated and grouped according to fabric type for each cat (i.e., all wools together, all cotton denims together, etc.).

Fel d 1 Extraction from Fabric

The extraction procedure was modified from a previously published study (Liccardi et al. 1998). The published procedure utilized Bovine Serum Albumin (BSA) in the extraction buffer. Preliminary experiments determined that Fel d 1 could be removed from fabrics by elution in 10 mL of either PBS-Tween or PBS-Tween/1% BSA. To determine a suitable extraction buffer, 100ng of commercially available, liquid, purified Fel d 1 (Indoor Biotechnologies) was seeded on eight wool squares and eight polyester knit squares. Fel d 1 on four of the wool and four of the polyester knit squares was extracted by immersion in 10 mL of 0.01 M phosphate buffered saline with 0.05% tween 20 (PBS-T). The remaining fabric squares were extracted using 10 mL of 0.01 M phosphate buffered saline with 0.05% tween 20 and 1% Bovine Serum Albumin (PBS-T/1% BSA) in a 50 mL conical tube. Previous studies have demonstrated washing is an effective means of removing Fel d 1 from fabrics (Liccardi et al. 1998). To simulate washing, the conical tubes were briefly vortexed, left to sit for 30 minutes, vortexed again, and allowed to sit for another 30 minutes. The fabric samples were removed and

the effluent analyzed via ELISA. No difference was noted between the two extraction buffers (Figure 2). PBS-Tween was chosen as the extraction buffer as it is stable at room temperature.

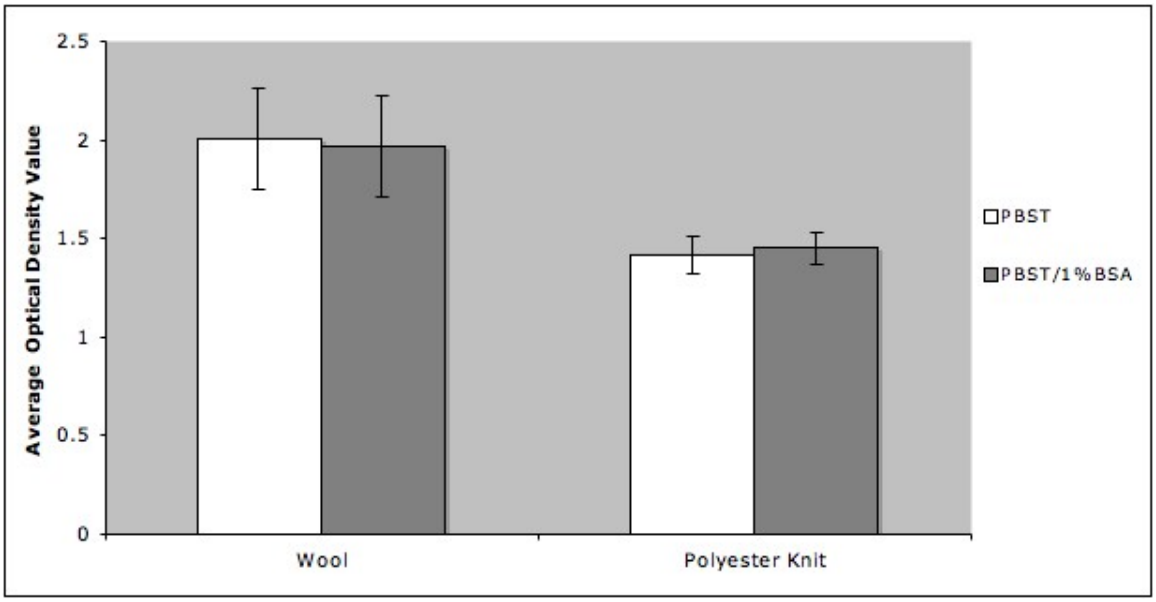

Figure 2: Evaluation of Extraction Buffers

Eight wool squares and eight polyester knit squares were seeded with 100ng of commercially available, liquid, purified Fel d 1. Four squares of each fabric were extracted using PBS-Tween and four squares of each fabric were extracted using PBS-Tween/1% BSA. Extracted Fel d 1 was analyzed via ELISA. Values shown are the average Optical Density (OD) values. Error bars represent the standard error for the samples. The data indicated that both buffers extract similar levels of Fel d 1 from the fabrics.

Prior to collection of Fel d 1 directly from cats, experiments were conducted to determine Fel d 1 stability on fabrics prior to extraction. 100ng of liquid, purified Fel d 1 was seeded onto samples of all fabric types and extracted as noted above after either an overnight or a 1 week drying time.

Extracting fabrics after 24 hours from seeding with Fel d 1 yielded results that suggest the Fel d 1 is stable on the fabric for 24 hours after seeding (Figure 3). The variability observed is within the range of the assay and is also observed with the positive control when Fel d 1 is analyzed without seeding or extracting from fabric (Figure 3).

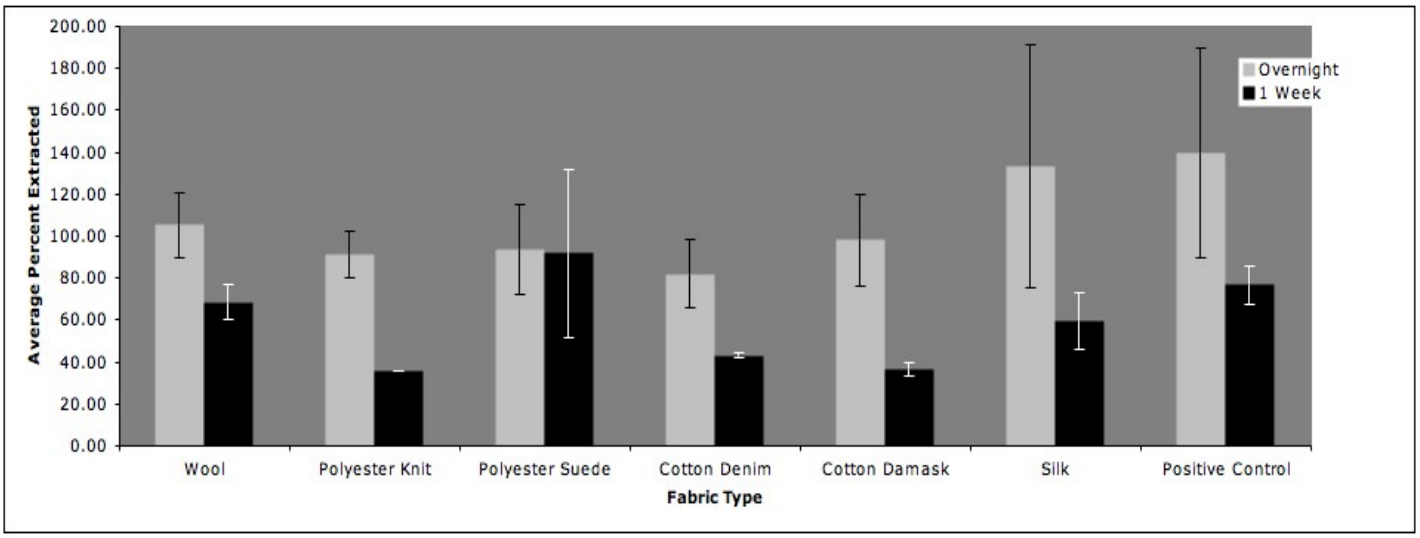

Figure 3: Evaluation of Extraction Buffers

Samples of each fabric type were seeded with 100ng of commercially available, purified, liquid Fel d 1 and incubated at room temperature for either overnight or one week before extraction. For each fabric type in each series, n=2 and samples were each assayed via ELISA in duplicate. Bars represent the average percent of Fel d 1 extracted and analyzed from each fabric type and error bars represent the standard error.

In contrast to the overnight extraction experiments, extracting fabrics 1 week after seeding with Fel d 1 yielded results that suggest Fel d 1 stability is reduced compared to overnight extraction (Figure 3). Therefore, with one exception, samples collected directly from cats were extracted within 24 hours after collection. For cat 30 samples were extracted and analyzed 48 hours after collection.

It should be noted extraction efficiencies over 100% are occasionally observed in ELISAs. Since samples are spread over a 96-well plate, small variances in time substrate or stop solutions are added can create small variances in the optical density (OD) value when read by spectrophotometer. Fitting these values to the standard curve can create what appear to be extraction efficiencies over 100%. As these experiments focused on determining if the amount of Fel d 1 extracted from fabrics over time diminishes, these small variances during the ELISA are less important.

It should also be noted the extraction process in these experiments may not be directly comparable to direct Fel d 1 collection from cats as the liquid antigen could potentially seep into the fabrics and embed in the fibers. Also, two of the fabrics, the polyester suede and the cotton damask, failed to absorb Fel d 1 quickly and left a residue.

Once Fel d 1 had been collected from cats, the quilt-squares were brought back to the laboratory and pulled apart. Since the Latin-square design was not the experiment, but merely a way to collect Fel d 1 evenly on all fabric types, the samples from one quilt-square were grouped together by fabric type. The grouped fabrics were cut in half, with half of each group saved for retention studies. The fabrics were extracted using the extraction procedure described above. Fabrics were removed from the conical tubes using clean forceps and pressed against the side of the tube to remove excess fluid. Once extracted, the Fel d 1 in the PBS-T buffer was analyzed by ELISA and diluted as necessary.

Evaluation of Fel d 1 Collected from Cats

A pilot study with three cats was performed to determine feasibility and set parameters for Fel d 1 collection. The cats tolerated the procedure well and the samples yielded quantifiable results (Table 2). Variability was noted between cats and between fabrics. It was unclear why there was a large variability in Fel d 1 production between cats, however the most obvious difference between the cats was that cats 1 and 3 were male and cat 2 was female. However, when the possibility of a sex specific difference in Fel d 1 production was tested across a larger sample of 40 cats no differences were observed (Figure 6). Therefore, variability in Fel d 1 production is not related to the sex of the animal.

Table 2: Total Fel d 1 Collected (ng) During Pilot Study

	Cat 1	Cat 2	Cat 3
Wool	3674	247	16945
Poly Knit	30929	346	23709
Poly Suede	13447	219	19971
Cotton Denim	11130	201	13761
Cotton Damask	14400	235	21543
Silk	4104	191	9898
Total	77683	1438	105826

The total amount of Fel d 1 collected from 3 cats in the pilot study, as determined by extraction from fabrics and analysis via ELISA. The values presented are in total amount of nanograms collected.

The initial study demonstrated that collecting Fel d 1 from cats was feasible and the study was expanded to an additional 37 cats (total = 40 cats) using the methods outlined above. Due to the variability in Fel d 1 production observed in the preliminary study, additional information was collected about the cats including age, sex, coat length, and the use of flea medications to determine if these factors might contribute to overall Fel d 1 production.

Intact males have been reported to produce higher levels of Fel d 1 than spayed females and neutered males (Jalil-Colome et al. 1996; Ramadour et al. 1998). However, intact males are less common in households than spayed females and neutered males and therefore only one cat in the study was an intact male (cat 27).

Fel d 1 Retention on Fabrics Over Time

One half of each fabric tested was stored in a closed plastic bag in the dark at ambient temperature for 14 weeks to test for degradation of Fel d 1 on different fabrics through time. The Fel d 1 on stored samples was extracted and quantified in the same manner as samples tested immediately after collection.

Data Analysis

Fel d 1 values obtained by ELISA were estimated by a linear regression of a standard curve of known dilutions of Fel d 1. Data were $\log_{10}$ transformed to meet assumptions of parametric statistics. To determine the effect of fabric on Fel d 1 collection, Fel d 1 levels were compared by analysis of variance in which cats were treated as blocks (because each fabric was fully replicated for each cat in the Latin square fabric rectangle). Fabrics and cats were analyzed as fixed factors. To determine if Fel d 1 levels decline over time, Fel d 1 levels at 14 weeks were compared to time 0 levels using a paired t-test. A paired t-test was used to determine if Fel d 1 levels decrease over time for all fabrics and if Fel d 1 levels decrease on different fabrics in varying amount. Of the 40 cats in the study, 36 were included in the paired t-tests. Two of the excluded animals had Fel d 1 levels that could not be accurately quantified after 14 weeks. One sample was too low to analyze, and another other produced variable results. In addition, two samples were lost. Other variables (e.g., sex, age, health status, coat length, use of flea medications) were evaluated for effects on Fel d 1 levels at the time of collection by plotting data using box plots, using the total amount of Fel d 1 collected and analyzed after initial collection. These potentially important modifiers of Fel d 1 levels were not otherwise analyzed (e.g., analysis of covariance) because they were not independent of the block (cats) effects. To analyze these potential effects more definitively will require a different study design. All statistical analyses were conducted with SPSS (v.17).

RESULTS

Fabrics Retain Different Fel d 1 Levels

Fel d 1 data that were $\log_{10}$ transformed met the assumptions of parametric statistics (Appendices A & B). Fel d 1 differed significantly among 40 cats in the study ($p<0.001$;Table 3), but the experimental design partitioned variation among cats separately from that among fabrics. Fel d 1 levels also were significantly different among fabrics ($p<0.001$; Table 3).

Table 3: Fel d 1 Levels Collected Differ Among Cats and Across Fabric Types

Dependent Variable: Log Amount of Fel d 1 Collected/ mL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	64.026[a]	44	1.455	58.535	.000
Intercept	1329.443	1	1329.443	53479.410	.000
Fabric	7.728	5	1.546	62.171	.000
Cats	56.298	39	1.444	58.069	.000
Error	4.847	195	.025		
Total	1398.316	240			
Corrected Total	68.873	239			

a. R Squared = .930 (Adjusted R Squared = .914)

Silk had significantly less Fel d 1 than other fabrics (Tukey's HSD; $p<0.001$; Figure 4). Wool, cotton denim and cotton damask had intermediate Fel d 1 levels and were not significantly different from each other but were significantly different from silk and both polyester fabrics (Tukey's HSD; $p<0.001$; Figure 4). Polyester knit and suede fabrics were not significantly different from each other but were significantly greater in Fel d 1 levels than all the other fabrics tested (Tukey's HSD; $p<0.001$; Figure 4).

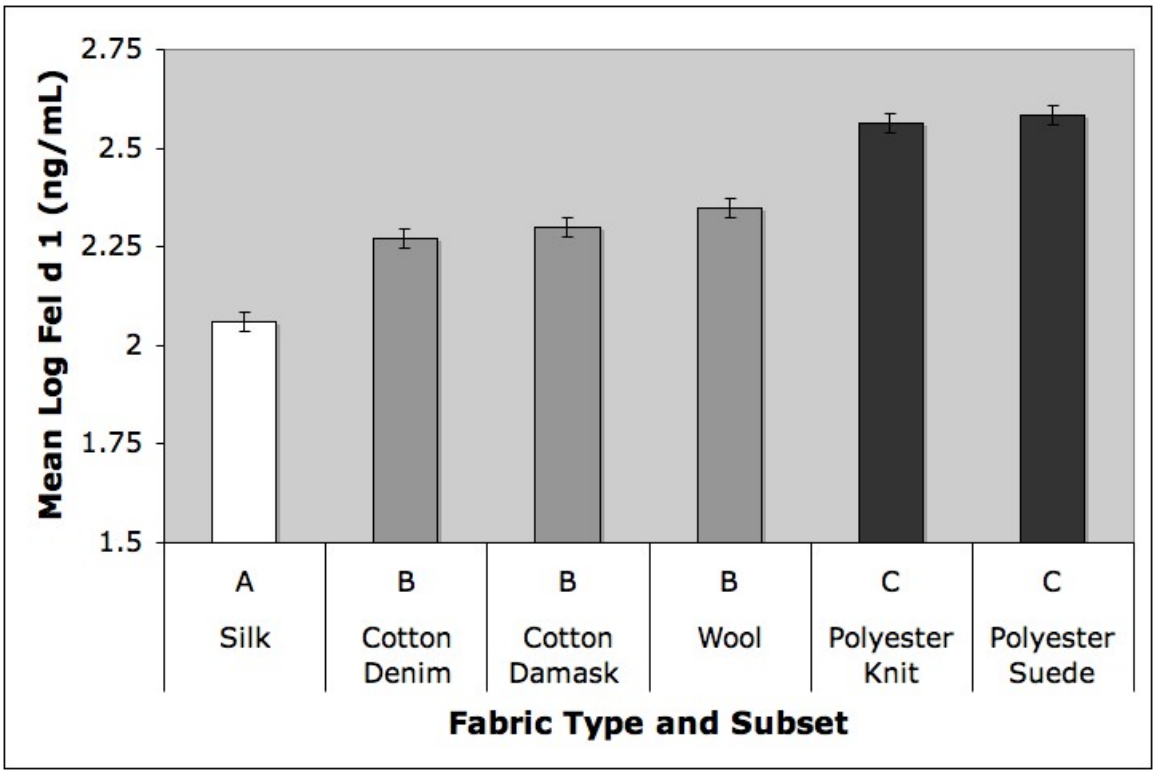

Figure 4: Relative Fel d 1 Collection on Fabrics

Tukey HSD subsets of Fel d 1 collected on fabrics. The bars represent the mean log value of Fel d 1 collected on fabrics and the error bars are the standard error. Fel d 1 was collected from 40 cats.

Recovery of Fel d 1 Diminishes Over Time

Fel d 1 levels significantly decreased on all fabrics combined ($p<0.001$) during the 14 weeks that fabric samples were stored (Figure 5). Fel d 1 levels also significantly decreased on fabrics when tested by type, however the decreased amount of Fel d 1 was barely significant for several fabric types (wool $p=0.047$; polyester knit $p=0.30$; polyester suede $p=0.37$; cotton denim $p=0.48$; cotton damask $p=0.001$; silk $p=0.002$; Figure 5). Fel d 1 is still present in levels high enough to cause an allergic reaction in sensitive individuals. The reduction in Fel d 1 is promising and further testing may determine how much time is necessary for Fel d 1 to diminish to levels that would not cause an allergic reaction in sensitive individuals.

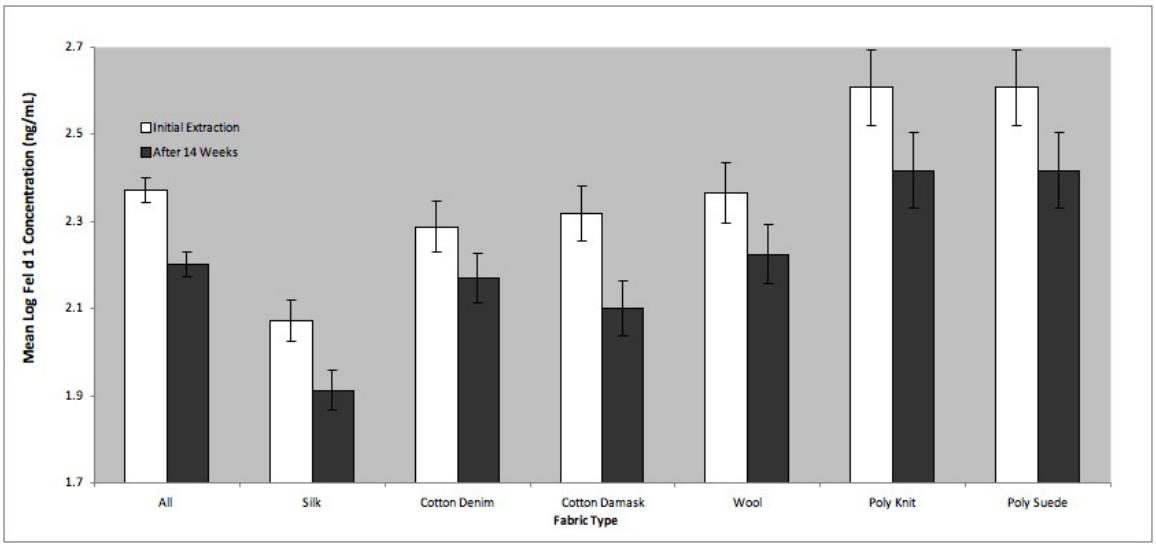

Figure 5: Fel d 1 Released from Fabrics

Paired T-test was used on all fabrics together and each fabric type to determine if Fel d 1 levels released from fabrics decreased after 14 weeks. The bars represent the mean levels of Fel d 1 extracted from fabrics (log transformed) and the error bars represent the standard error.

Fel d 1 Production Levels Do Not Differ Between the Sexes

Twenty spayed females and 19 neutered males (1 cat was not neutered) were compared for Fel d 1 levels (Figure 6). Both data sets overlap almost completely, indicating no significant difference between neutered males and spayed females in Fel d 1 production. This finding supports previous research that demonstrated neutered males and females produce Fel d 1 in similar amounts (Ramadour et al. 1998; Nicholas et al. 2008).

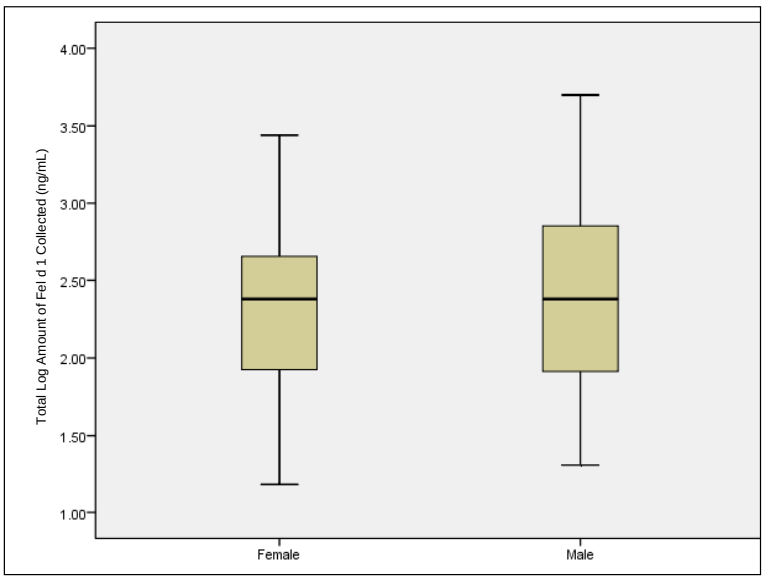

Figure 6: Sex of Cat vs. Total Amount of Fel d 1 Collected

The total amount of Fel d 1 collected from males and females were graphed using a box plot (n=20 for females and n=19 for males). The upper quadrant of the box represents the upper 25% of samples, the line in the middle of the box represents the median of the sample set, and the lower quadrant of the box represents the lower 25% of samples. The whiskers represent the upper and lower values of the samples.

Flea Medications May Reduce Amount of Fel d 1 Collected from Cats

Among the 40 cats tested in this study, 12 used topically applied flea medications (typically applied monthly to the back of the animal's neck) and 28 did not. Based on my results (Figure 7), topical flea medications may reduce Fel d 1 levels on cats compared to cats that were not dosed, however the sample size was limited (data from 5 cats using flea medication are outliers).

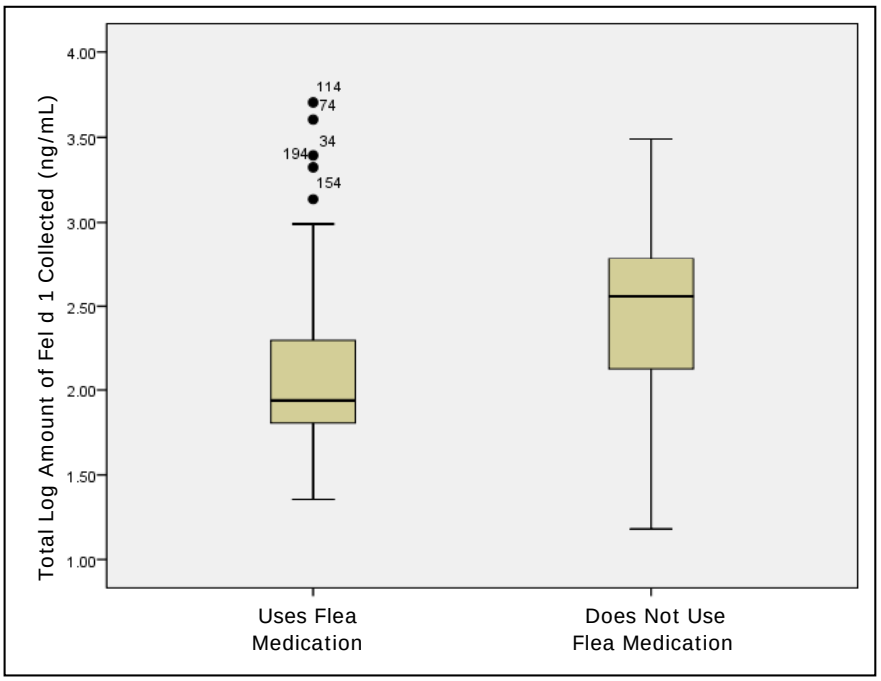

Figure 7: Flea Medication Usage vs. Total Amount of Fel d 1 Collected

The total amount of Fel d 1 collected from cats dosed with flea medication and cats not dosed with flea medication were graphed using a box plot (n=12 for cats using flea medication and n=28 for cats not using flea medication). The upper quadrant of the box represents the upper 25% of samples, the line in the middle of the box represents the median of the sample set, and the lower quadrant of the box represents the lower 25% of samples. The whiskers represent the upper and lower values of the samples and the small dots represent outliers in the data.

Reduced levels of Fel d 1 on treated cats may indicate reduced Fel d 1 secretion following treatment, or interference of the ELISA assay by flea medications. Additional possibilities include degradation of Fel d 1 on cats by the flea medication or altered fabric-Fel d 1 interactions in treated animals. Further testing may be required to determine what effect, if any, flea medications have on Fel d 1 production, collection and extraction.

DISCUSSION

Fel d 1 Extracted from Fabric

The silk, wool and cotton fabrics used in this study are all made of natural fibers, whereas polyester is a synthetic fiber that is popular in many types of applications. The results of this study indicate that silk dupioni is a good option to use in homes of those suffering from cat allergies. Silk dupioni is a thin, woven fabric with a fairly tight weave. Unfortunately, silk dupioni is not a very versatile fabric and its use is primarily restricted to draperies. However, the slippery nature of this fabric most likely contributes to its ability to resist adhesion of Fel d 1.

The wool suiting and both cotton fabrics tested would track Fel d 1 from the homes of cat owners into environments with no cats however they are preferable over the polyester fabrics tested. Both cotton damask and polyester suede are commonly used in home upholstery. This study indicates that the cotton damask is less likely to collect Fel d 1 than polyester suede. The polyester suede has a nap, that is, when brushed in different directions, the fibers appear to be slightly different colors when viewed from various angles. The other polyester fabric used in this study is a knitted fabric. A knitted fabric has one continuous thread looped through itself that produces a flat surface that is structurally different, and in some cases more flexible, than a woven fabric.

Of the fabrics tested in this study, the polyester suede and polyester knit fabrics collected the most Fel d 1. Given that both polyester fabrics bound Fel d 1 similarly despite having different fabric designs, it is likely that elevated Fel d 1 binding on polyesters is due to the molecular construction of the polyester fibers.

A previous study by Patchett et al. (1997) also found that Fel d 1 adheres to polyester

fabrics in higher levels compared to cotton fabrics. However, Patchett et al. determined Fel d 1 adhered to wool in levels similar to polyester, whereas my study indicates that Fel d 1 adheres to wool in similar levels as cotton. Patchett's study aimed to determine if Fel d 1 was carried into schools on the clothing of children and therefore tested the clothing worn by children. Higher levels of Fel d 1 for the wool fabrics in their study may be a result of infrequent washing of these garments, allowing Fel d 1 levels to accumulate. My study demonstrates that when applied to non-contaminated fabrics directly, Fel d 1 adheres to polyester knit and polyester suede in higher levels than cotton denim, cotton damask and wool and that all of these fabrics adhere Fel d 1 in higher levels than silk dupioni.

My research supports previous findings that Fel d 1 is carried on fabrics (D'amato et al. 1997; Patchett et al. 1997; Ritz et al. 2002; Karlsson et al. 2004). When presented with the fabric options included in this study, an individual with sensitivity to Fel d 1 may wish to choose cotton or wool clothing and cotton or silk home fabrics instead of the polyester options. Further research should focus on determining if Fel d 1 adhesion to natural fibers differs from synthetic fibers and if fabric construction contributes to Fel d 1 adhesion. Many synthetic fibers, such as polyester, spandex, and nylon are often blended with cottons into fabrics that take on the properties of the blended materials (i.e., soft cotton blended with a strong, stain and water resistant synthetic fiber). Further research can focus on determining how Fel d 1 adheres to blended fabrics.

Retention of Fel d 1 on Fabrics

I extracted Fel d 1 from fabrics but did not determine if some of the Fel d 1 was permanently retained. Due to the nature of the collection method, the Fel d 1 presumably

did not forcibly embed into the fibers of the fabric, but instead settled on the surface. However, this differs from a real-life scenario where a cat may lie on the surface of a couch or rub against clothing, actively depositing and rubbing in Fel d 1, and a mixture of fur, skin and other microbes, on the surface. In this scenario, the protein may become embedded into the fibers of the fabrics over time. While the fabrics may permanently retain some of the Fel d 1, the Fel d 1 released from the fibers is of more concern to those sensitive to the allergen. When the allergen is released from the fabric, it can aerosolize and be inhaled, causing an allergic reaction in sensitive individuals. The Fel d 1 that is permanently embedded in the fibers poses no such risk.

Variant Forms of Fel d 1

Previous studies demonstrated that Fel d 1 production varies across the body of cats and the Fel d 1 protein shows variability depending on the site of production (Caroyol et al. 2000; Bienboire-Frosini et al. 2009). In addition to the intact form of Fel d 1, truncated versions of the protein are produced (Griffith et al. 1992) and truncated versions of the allergen have been demonstrated to cause allergic reactions in those individuals sensitive to Fel d 1 (Smith et al. 2004). I was concerned that variant forms of Fel d 1 might not possess the epitopes necessary for binding of the antibodies used in this study. However the antibodies in this study react strongly with the Fel d 1 variants collected from the face and chest and have been demonstrated to react with both the intact and truncated forms of the protein (Bienboire-Frosini et al. 2010). The strong interaction between the antibodies and the various forms of Fel d 1 is because the antibodies used react with sites on the α-chain that are present in all known variants of the protein. The data presented in this paper are valid for Fel d 1 collected from different anatomical sites and for known

truncated and intact forms of the protein.

Fel d 1 Degradation Over Time

While the ELISA antibodies used are specific to two epitopes on the α-chain, the ELISA may not detect degraded Fel d 1 if, for example, the protein degrades in such a way that the epitopes are separated. The data in this paper show that the amount of Fel d 1 that can be extracted from all fabrics diminishes, over 14 weeks. It is possible that the reduction in signal is due to protein degradation and the methods used cannot detect the degraded protein. Previous research has demonstrated that the antibodies used in this study will not detect severely degraded Fel d 1 (Bienboire-Frosini et al. 2010). There is no evidence to suggest Fel d 1 degraded in this manner would cause allergic reactions in sensitive individuals. Further, it is also possible that microbes present on the fabric or introduced during Fel d 1 collection may be degrading the protein. Alternately, it is possible that Fel d 1 is adhering more strongly to the fibers and is not removed by the extraction procedure. Further research is required to determine if there is long-term retention of Fel d 1 on fabrics, or if Fel d 1 is being degraded. Mass spectrometry may be able to determine if degraded Fel d 1 is being extracted from the fabrics or if the Fel d 1 is not being removed from the material. For all cats in this study, and across all fabrics, the levels of Fel d 1 extracted and analyzed 14 weeks after collection were at levels more than sufficient to cause an allergic reaction in sensitive individuals. It may take longer than 14 weeks for Fel d 1 levels to diminish to levels potentially low enough to not cause an allergic reaction and further research may focus on determining how much time is necessary.

Over time, Fel d 1 levels extracted from fabrics did diminish, however levels did not

diminish low enough not to cause an allergic reaction in sensitive individuals. Understanding how much time is necessary for Fel d 1 levels to further diminish would be beneficial for those in cat-free environments that can not be fully cleaned (i.e., work places, school, etc) and to determine how long to wait until Fel d 1 is no longer at levels high enough to cause an allergic reaction. While all fabrics did show significantly diminished levels of Fel d 1 after 14 weeks (Figure 5), the cotton denim, cotton damask and wool levels were barely significant. These fabrics may be binding the allergen in such a way that the protein is more easily released after 14 weeks, and may not be good choices for fabrics that cannot be frequently laundered when controlling for the amount of allergen in an indoor environment.

Variability in Fel d 1 Collection Among Cats

The results in this study indicate that the Fel d 1 collected varies wildly among cats (Table 3). While I attempted to determine why this variability in Fel d 1 collected occurred, I cannot conclusively identify any factor contributing to this significant difference among cats. This finding is intriguing, and further research should focus on determining why more Fel d 1 is collected on some cats compared to others. Identifying factors that contribute to the amount of Fel d 1 produced by cats may be useful for those suffering from cat allergies or those with loved ones who suffer from cat allergies chose specific cats as pets.

Sex Differences

Previous research indicated that females and neutered males produce similar levels of Fel d 1, while intact males produce significantly more (Zielonka et al. 1994; Miller et al. 1995; Ramadour et al. 1998). My results show that females and neutered males

produce similar levels of Fel d 1. Only one cat in this study, cat 27, was an intact male, but he did not produce significantly more Fel d 1 than the neutered males in this study.

Use of Flea Medication

My data indicate that the use of flea medication may reduce the amount of Fel d 1 extracted and analyzed from cats. This potential effect of flea medicines on Fel d 1 levels is intriguing, and more research should be conducted to determine if the chemicals in flea medications are inhibiting the ELISA assay or inhibiting Fel d 1 production by cats. Alternatively, the chemicals in flea medicines may be degrading the Fel d 1 on the animal's skin or on the fabric. These results are preliminary and this potential effect of flea medication as a method of controlling Fel d 1 levels should be studied further.

Conclusion

While all fabrics in this study collected Fel d 1 at levels that would cause a reaction in sensitive individuals, this research does suggest that Fel d 1 interacts with fabrics differently and the choice of fabrics in home or clothing can affect the amount of Fel d 1 carried into other environments. Ultimately, utilizing fabrics that are less likely to act as reservoirs or carriers of Fel d 1 will help those that suffer from allergies to control their symptoms. In conjunction with other allergy reduction measures, such as minimizing fabrics in the home and limiting exposure to cats, the use of fabrics that are less likely to collect Fel d 1 can be a useful component in controlling allergen exposure. Using fabrics that are less likely to collect and disperse Fel d 1 may also minimize the amount of Fel d 1 tracked into non-cat environments, thereby lessening the amount of Fel d 1 in workplaces, schools and other non-cat environments. Wearing fabrics that are more resistant to Fel d 1 adhesion may also minimize the amount of Fel d 1 carried back to the

home of individuals sensitive to cat allergens after visiting locations where the allergen is present. This study provides a basis for future research on the use of fabrics as a practical, non-pharmaceutical approach to controlling exposure to Fel d 1. This study also determined that cats may be producing a large variation of Fel d 1. Determining what factors may contribute to this variation would beneficial to determining why cats are producing this protein. This information, in turn, could be beneficial to those suffering from allergies to cats limit Fel d 1 exposure.

APPENDIX A: AMOUNT OF FEL D 1 EXTRACTED AND ANALYZED IMMEDIATELY AFTER COLLECTION (NG/ML)

ng/ml	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8	Cat 9	Cat 10
Wool	367.37	24.70	1694.47	112.14	62.91	612.15	88.33	75.78	106.44	74.08
Poly Knit	3092.91	34.56	2370.94	275.76	96.70	824.25	103.64	122.69	259.25	79.11
Poly Suede	1344.67	21.93	1997.06	189.00	77.30	912.87	175.79	104.79	180.94	154.24
Cotton Denim	1113.00	20.12	1376.10	118.48	59.99	664.56	97.61	68.88	83.93	70.77
Cotton Damask	1439.95	23.45	2154.25	75.27	58.58	490.28	96.53	64.16	65.89	85.83
Silk	410.43	19.06	989.78	61.78	51.98	244.17	70.12	51.50	51.16	67.31
Total amount (ng/ml)	7768.33	143.82	10582.60	832.43	407.46	3748.27	632.01	487.80	747.60	531.34

ng/ml	Cat 11	Cat 12	Cat 13	Cat 14	Cat 15	Cat 16	Cat 17	Cat 18	Cat 19	Cat 20
Wool	98.46	60.21	73.45	416.43	606.01	282.14	214.59	448.01	498.24	260.96
Poly Knit	158.26	94.73	86.20	346.09	545.41	391.53	449.81	396.26	475.93	387.65
Poly Suede	104.55	86.45	99.59	299.71	609.13	789.64	451.54	439.88	454.63	655.81
Cotton Denim	82.70	56.30	78.21	312.29	393.54	401.20	167.47	333.58	431.89	173.76
Cotton Damask	81.06	59.27	73.55	282.14	419.66	395.06	163.43	328.12	378.52	122.46
Silk	69.96	60.12	60.73	278.90	386.53	114.63	60.84	179.92	384.11	59.28
Total amount (ng/ml)	594.98	417.08	471.73	1935.57	2960.28	2374.21	1507.68	2125.76	2623.32	1659.92

ng/ml	Cat 21	Cat 22	Cat 23	Cat 24	Cat 25	Cat 26	Cat 27	Cat 28	Cat 29	Cat 30
Wool	357.62	133.15	42.56	44.79	235.05	366.33	821.07	623.81	457.19	312.16
Poly Knit	480.31	309.40	141.68	99.34	1004.73	411.44	712.36	1258.90	687.63	376.51
Poly Suede	675.32	227.79	263.13	119.43	1347.79	421.43	763.32	1327.38	756.75	538.85
Cotton Denim	103.84	87.15	75.62	21.97	125.62	432.49	910.74	570.38	264.39	176.08
Cotton Damask	507.45	41.54	64.02	27.25	216.41	447.69	467.90	556.53	290.36	231.34
Silk	110.11	22.87	35.88	15.14	102.18	338.29	722.00	211.61	157.09	46.50
Total amount (ng/ml)	2234.66	821.90	622.90	327.92	3031.78	2417.67	4397.39	4548.61	2613.40	1681.45

ng/ml	Cat 31	Cat 32	Cat 33	Cat 34	Cat 35	Cat 36	Cat 37	Cat 38	Cat 39	Cat 40
Wool	183.64	1174.91	83.24	2495.47	95.81	663.48	521.89	232.79	20.43	1529.80
Poly Knit	205.07	2384.31	287.45	3994.45	144.22	951.09	733.36	773.36	28.14	2717.97
Poly Suede	574.88	1963.63	192.37	4998.96	206.34	979.19	569.96	637.57	28.06	2757.88
Cotton Denim	168.26	1297.11	84.40	1372.01	48.24	343.82	339.55	159.44	21.67	1485.85
Cotton Damask	281.43	1388.35	60.79	2134.84	63.72	572.28	314.27	246.18	24.09	1872.23
Silk	146.05	343.81	66.17	878.56	40.59	208.47	222.28	112.94	24.57	472.68
Total amount (ng/ml)	1559.33	8552.12	774.43	15874.29	598.92	3718.34	2701.30	2162.28	146.97	10836.40

APPENDIX B: AMOUNT OF FEL D 1 EXTRACTED AND ANALYZED 14 WEEKS AFTER COLLECTION (NG/ML)

ng/ml	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8	Cat 9	Cat 10
Wool	297.09	12.92	617.75	273.41	367.29	446.71	18.54	116.68	225.33	102.08
Poly Knit	314.40	15.20	539.65	327.31	437.16	552.93	281.24	142.39	339.59	312.07
Poly Suede	329.83	15.72	559.71	862.88	182.87	785.06	313.76	162.57	293.53	324.59
Cotton Denim	283.40	12.46	646.05	271.37	110.73	354.65	200.13	103.48	142.96	105.34
Cotton Damask	331.03	13.21	843.07	178.08	98.27	323.99	135.58	100.52	137.59	70.54
Silk	275.89	12.08	569.07	72.30	30.47	252.57	136.99	89.98	98.62	34.22
Total amount (ng/ml)	1831.63	81.59	3775.30	1985.35	1226.80	2715.92	1086.24	715.64	1237.62	948.83

ng/ml	Cat 11	Cat 12	Cat 13	Cat 14	Cat 15	Cat 16	Cat 17	Cat 18	Cat 19	Cat 20
Wool		78.06	302.67	369.74	627.04	259.54	168.13	343.70	302.78	222.43
Poly Knit		449.25	772.91	434.68	627.71	285.54	260.26	339.37	241.27	224.31
Poly Suede		511.27	550.68	413.94	644.50	431.66	315.02	377.97	215.26	247.47
Cotton Denim		39.80	433.06	452.62	348.17	180.89	84.69	304.39	377.18	176.31
Cotton Damask		37.70	220.60	267.06	386.55	138.33	169.66	265.89	273.59	99.25
Silk		44.74	85.41	282.34	121.72	148.00	40.74	39.30	143.49	49.85
Total amount (ng/ml)		1160.82	2365.34	2220.37	2755.70	1443.95	1038.50	1670.62	1553.57	1019.61

ng/ml	Cat 21	Cat 22	Cat 23	Cat 24	Cat 25	Cat 26	Cat 27	Cat 28	Cat 29	Cat 30
Wool	504.83	42.90	17.30	26.64	31.48	333.09	490.30			81.11
Poly Knit	386.04	107.77	41.32	43.23	48.17	288.84	460.30			175.41
Poly Suede	444.27	209.89	68.05	33.34	35.10	338.77	575.06			248.24
Cotton Denim	99.15	37.12	36.64	30.30	26.37	427.11	454.65			63.61
Cotton Damask	216.63	26.25	25.31	16.44	19.29	320.96	365.58			55.95
Silk	49.80	26.60	19.97	14.85	19.16	309.27	445.28			33.74
Total amount (ng/ml)	1700.71	450.53	208.59	164.80	179.57	2018.04	2791.17			658.07

ng/ml	Cat 31	Cat 32	Cat 33	Cat 34	Cat 35	Cat 36	Cat 37	Cat 38	Cat 39	Cat 40
Wool	325.15	287.70	73.57	93.94	50.44	266.24	688.53	272.39		606.21
Poly Knit	313.77	279.10	85.77	104.29	60.20	311.41	911.26	334.79		1549.09
Poly Suede	361.33	230.70	52.17	102.20	59.70	457.79	760.22	581.33		1509.15
Cotton Denim	247.63	249.29	64.29	122.82	39.24	252.27	387.99	139.63		600.95
Cotton Damask	245.03	178.55	36.68	103.94	19.49	158.24	305.70	172.55		1227.89
Silk	208.48	170.61	29.61	96.03	16.19	131.30	162.52	98.72		609.18
Total amount (ng/ml)	1701.39	1395.95	342.09	623.23	245.26	1577.26	3216.23	1599.41		6102.47

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