

House dust and storage mite contamination of dry dog food stored in open bags and sealed boxes in 10 domestic households

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Conflict of Interest

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Abstract

Dry pet food is a potential source of exposure to house dust and storage mite allergens in canine atopic dermatitis. This study evaluated contamination of house dust and dry dog food stored in paper bags, sealable plastic bags and sealable plastic boxes in 10 households for 90 days using Acarex® tests for guanine, a Der p 1 ELISA and mite flotation. Acarex® tests were negative in all the food samples but positive in all the house dust samples. The Der p 1 levels and mite numbers significantly increased in food from paper bags ($P = 0.0073$ and $P = 0.02$, respectively), but not plastic bags or boxes. Mite numbers and Der p 1 levels were 10–1000 times higher in house dust than the corresponding food samples ($P < 0.0001$). There were significant correlations between Der p 1 in house dust and food from the paper ($P < 0.0001$) and plastic bags ($P = 0.003$), and mite numbers in house dust and food from the paper bags ($P = 0.0007$). Bedding and carpets were significantly associated with Der p 1 levels in house dust ($P = 0.015$ and $P = 0.01$, respectively), and food from the paper (both $P = 0.02$) and plastic bags ($P = 0.03$ and $P = 0.04$, respectively). Mites were identified in six of 10 paper bag, three of 10 plastic bag, one of 10 plastic box and nine of 10 house dust samples. These comprised *Dermatophagoides* (54%), *Tyrophagus* (10%; all from food) and unidentified mites (36%). Storage of food in sealable plastic boxes largely prevented contamination for 3 months. Exposure to mites and mite proteins in all the stored food, however, appeared to be trivial compared with house dust.

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Introduction

Canine atopic dermatitis (cAD) is a common pruritic and inflammatory dermatosis characterized by the presence of IgE specific to environmental allergens.¹ The most frequently implicated allergens are *Dermatophagoides* species house dust mites (HDM).² There is ample evidence from intradermal tests (IDTs), epicutaneous patch tests, IgE serology, immunoblots, peripheral blood mononuclear cell proliferation, exacerbation following exposure, amelioration after avoidance and responses to allergen-specific immunotherapy, that they are relevant in cAD.^{2–10} Several studies have also shown that they are abundant in domestic environments, particularly carpets, bedding and dogs' coats.^{11–13}

Sensitivity to storage mites, such as *Tyrophagus*, *Acarus* and *Lepidoglyphus* species, is also common in atopic dogs.^{2,14,15} Storage mites are considered relevant allergens in humans,^{16–19} but their relevance to cAD is more controversial. Most dogs appear to be exposed to these mites, although they are usually considered to be rare in 'true' house dust and are mainly seen in stored foodstuffs.^{2,20} An early paper, however, found that all house dust samples from a range of British homes were contaminated with both house dust and storage mites.²¹ Other studies have also reported that domestic homes contain mixed mite populations, up to 8% of which may be storage mites such as *Acarus*, *Glycyphagus*, *Lepidoglyphus* and *Tyrophagus*.^{22–24} Immediate-type hypersensitivity has been demonstrated by passive cutaneous transfer in dogs,²⁵ and immunoblotting studies revealed that 95% and 89% of atopic dogs had serum IgE against high-molecular weight proteins from *A.* and *T. putrescentiae*, respectively.²⁶ It has also been shown, however, that one-third of both healthy and atopic dogs have positive IDTs to *T. putrescentiae* at 1000 PNU/mL.²⁷ Most storage mite sensitization, furthermore, appears to be associated with sensitization to *Dermatophagoides* species. Unpublished observations by the authors of 510 IDTs, for example, revealed significant (all $P < 0.0001$) odds ratios for co-sensitization to *T. putrescentiae*, *A. siro* and *L. destructor* of 30.3, 28.3, 29.1, respectively, in atopic dogs positive to *D. pteronyssinus*. Studies of IDTs, IgE serology and *in vitro* cross-inhibition in mite-sensitized dogs with spontaneous cAD, high-IgE responder beagles experimentally sensitized to *D. farinae* only and healthy beagles demonstrated extensive cross-reactivity among house dust and storage mites.²⁸ A majority of the dogs experimentally sensitized to *D. farinae*, moreover, also had positive IDTs and serology to *D. pteronyssinus*, *A. siro*, *T. putrescentiae* and *L. destructor* despite a lack

of specific exposure to these species. Nevertheless, monosensitization to storage mites has been reported in atopic dogs.¹⁵ It is therefore possible that exposure to storage mites may exacerbate cAD through both cross-reaction with *Dermatophagoides* species and specific sensitization.

The most likely route of exposure to storage mites is through contact with stored foodstuffs, including dry pet food. Oral, epicutaneous and inhalation exposure of specifically sensitized beagles to *D. farinae* extracts results in pruritus and lesions typical of generalized cAD,⁷ although the different routes of exposure had additive effects and did not determine the distribution of lesions. A more recent study found that oral and epicutaneous exposure of *D. farinae*-sensitized beagles to *T. putrescentiae* exacerbated the clinical signs of cAD.²⁹ House dust mite and storage mite contamination of dry pet foods could therefore be an important source of allergens through direct contact, inhalation and ingestion. Contaminated pet food may also pose a risk to sensitized humans.

Both *A. siro* and *T. putrescentiae* can be found in dry pet food and associated factories,³⁰ and could therefore contaminate food at source.²⁴ *Tyrophagus putrescentiae* has recently been shown to survive and proliferate on three different commercial dry dog foods, although this was enhanced by the presence of mould and moisture.³¹ An early *in vitro* laboratory study, furthermore, demonstrated that *D. farinae* can survive and reproduce on a substrate of dry dog food.³² Despite this, weekly direct microscopy and mite flotation of 23 samples of dry commercial food in Germany for 6 weeks failed to detect any storage mites.³³ A US study using a specific group 2 *Dermatophagoides* allergen ELISA found no evidence of contamination in 30 newly opened bags and 50 samples of home-stored commercial dry foods.³⁴ Both of these studies were performed in temperate continental climates, where *D. farinae* is the predominant HDM species. In contrast, a study in Spain (with a warm Mediterranean climate) using direct microscopy, guanine tests (Acarex®; Allergopharma, Reinbeck, Germany), storage mite traps and flotation concluded that dry dog food supports storage mite survival and proliferation.³⁵ *Acarus siro* was found in one of 10 newly opened bags of different foods, and storage mites (predominantly *Tyrophagus*) were found in nine of 10 bags stored for 5 weeks in a garage at 23.2 ± 2.1 °C and $71 \pm 5.6\%$ relative humidity. No mites, however, were seen in the same food samples stored in a laboratory at 16 ± 2.3 °C and $68 \pm 7.3\%$ relative humidity for the same period.

The potential for contamination of dry pet foods therefore appears to vary with climatic, environmental and storage conditions, and the significance of this compared with house dust has not been evaluated. The aim of this study was to assess HDM and storage mite contamination of a single type of dry dog food stored in three different ways in 10 dog-owning households in a temperate maritime climate (where *D. pteronyssinus* is the predominant HDM species^{11,12}) for 3 months. The degree of mite and allergen contamination, was, furthermore, compared with that in dust samples from the food storerooms.

Materials and methods

Food storage

The study was performed between April and August 2009. Households were selected from those known to the authors that were willing to store the food and be sampled, and had no specific allergen-avoidance measures in place.

Ten identical bags of food from a single batch were used in the study (Hill's™ Science Plan™ Canine Adult Light with chicken 3 kg). One bag was used for each household. The food in each bag was mixed and divided equally between the original bag (plastic with a Ziploc®-type closure), a paper bag (the top was rolled over to close) and a plastic box (with a locking lid and rubber seal).

The three food samples were stored adjacent to each other in the normal food storeroom in each household. The occupants were instructed to open each container once daily and record this on a diary sheet. A data logger (EL-USB-2; Lascar Electronics, Salisbury, UK) set to record temperature and relative humidity every 5 min was kept with the food samples. Demographic data for each household were obtained through a standard questionnaire.

Food and house dust samples

One-hundred gram samples of food were taken from each bag at day 0. Subsequently, 100 g samples were taken from food stored in the paper bags, plastic bags and plastic boxes on days 30, 60 and 90 (± 6 days). The food was thoroughly mixed before sampling on each occasion. Each sample was sealed in a plastic bag and stored at -20 °C before processing. Temperature and relative humidity data were downloaded on days 30, 60 and 90 (EasyLog USB; Lascar Electronics).

House dust samples were collected on day 90 (± 15 days) according to an established protocol.¹¹ Briefly, house dust was collected from the floor adjacent to the stored food by vacuuming four evenly spaced 21×30 cm (i.e. A4) areas in each food storeroom for 30 s each using a Mitest® filter (Indoor Biotechnology, Cardiff, UK) according to the manufacturer's instructions. The dust samples were placed in a sealed plastic bag and stored at -20 °C before processing.

Acarex® tests

Each food and house dust sample was agitated and mixed to obtain 100 mg of fine dust. The Acarex® tests were carried out according to the manufacturer's instructions. Briefly, each sample was mixed with the alkaline/alcohol extraction buffer for 60 s. A test strip was then placed in the sample solution and read after 60 s. The colour reaction was interpreted as follows: red, strongly positive; dark pink, moderately positive; pale pink, slightly positive; and no colour, negative.

Der p 1 quantification

The food and house dust samples were agitated and mixed to obtain 100 mg of fine dust. This was mixed with 2 mL 0.05% Tween 20 in phosphate-buffered saline, pH 7.4 (PBS-T) at room temperature for 2 h. The solutions were then centrifuged at 550 g at 4 °C for 20 min and the supernatants stored at -20 °C.

Polystyrene microtitre wells (Immulon II®; Thermo Electron Bioscience Technologies, Basingstoke, UK) were coated with 200 ng/well anti-Der p 1 (mAb 10B9; Indoor Biotechnology) and incubated at 4 °C for 16 h. Each well was blocked for 1 h at room temperature with 100 µL 1% bovine serum albumin (BSA) in PBS-T. One hundred microlitres of 10-fold dilutions from 250 to 0.5 ng/mL of purified Der p 1 (Indoor Biotechnology) were added to the appropriate wells. One hundred microlitres of the test samples were added at 1:10, 1:20 and 1:40 dilutions. Negative controls with PBS-T only were included, and all wells were in duplicate. The plates were then incubated for 1 h at room temperature before adding 100 µL of a 1:1000 dilution of biotinylated anti-Der p 1 antibody (mAbs 5H8; Indoor Biotechnology) to each well and incubating for a further hour at room temperature. One-hundred microlitres of 250 ng/mL streptavidin-peroxidase (Sigma, Poole, UK) was added to each well and the plates

were incubated at room temperature for 30 min. The assays were developed by adding 100 µL tetramethylbenzidine (Uptima TMB standard solution; Cheshire Sciences Ltd, Chester, UK) for 30 min at room temperature and then stopping the reaction with 100 µL 1 M sulphuric acid. The plates were washed three times with PBS-T between each step. All reagents were diluted in PBS-T containing 1% BSA. Optical density was read at 450 nm (Multiskan EX; Thermo Electron Bioscience Technologies). The purified Der p 1 solutions were used to construct a standard curve for each plate. Regression equations were then used to quantify the Der p 1 levels in each sample (Prism 4[®]; Graphpad Inc., San Diego, CA, USA).

Mite counts

Ten grams from each food sample and 1 g from each house dust sample were emulsified in 20 and 2 mL saturated NaCl (specific gravity 1.2), respectively. The emulsified solutions were made up to 50 mL with saturated NaCl and mixed for 5 min. The mixed solutions were then centrifuged at 220 *g* for 10 min. All the floating material was transferred to a Petri dish with a marked grid. The material in the Petri dish was methodically searched at $\times 40$ magnification. Individual mites were transferred to a microscope slide for identification to genus level at $\times 100$ magnification.^{36–38} The mite counts in the house dust samples were multiplied by 10 to ensure equivalence with the food samples.

Data analysis

The Der p 1 and mite count data sets were normally distributed (Kolmogorov–Smirnov tests; Instat[®]; Graphpad Inc) except for the following: Der p 1 levels in food from the plastic boxes on days 30, 60 and 90; and mite counts in food on day 0, food stored in the plastic bags and plastic boxes on days 30 and 60 and in the plastic boxes on day 90. The effects of the type of storage on Der p 1 levels and mite counts throughout the study, however, were analysed using a repeated-measures general linear model two-way ANOVA, as the distribution of the data prevented computation of nonparametric repeated-measures Friedman tests (Minitab 15[®]; Minitab Inc., Coventry, UK). Friedman tests (Minitab 15[®]) were used to compare Der p 1 levels and mite counts in the food samples and house dust at each site on day 90. Pearson and Spearman rank tests (Instat[®]) were used to correlate normal and non-normal data, respectively. The temperature and relative humidity data were not normally distributed, although the data for each household and time point were again tested with a repeated-measures general linear model two-way ANOVA (Minitab 15[®]). Associations between relative humidity, temperature and continuous demographic data with Der p 1 levels and mite numbers throughout the study were analysed by multiple regression (Minitab 15[®]). Nominal (where there was no implicit ordering of categories) or ordinal (implies ordering of categories) logistic regression tests (Minitab 15[®]) were used to compare the Acares[®] test results with the Der p 1 levels, and Der p 1 levels and mite numbers with the categorical demographic data.

Results

Food storage

The food containers were opened on a mean of 81 days (range 67–88 days) in the study. There was no significant difference between the households (data not shown). All the paper bags had at least one hole by day 90, and oil from the food had soaked through the paper in all cases. One plastic bag had a hole by day 90, and four of 10 closures had failed (there was no significant association with Der p 1 levels or mite numbers; data not shown). The plastic boxes remained intact throughout the study.

Demographic data

The demographic data for each household are shown in Table 1. Most were either insufficiently discriminatory for

analysis or not significantly associated with Der p 1 levels and mite numbers (data not shown).

The presence of pet bedding in the food storeroom was significantly associated with mite numbers in food from the paper bags (logistic regression, $P = 0.007$), and Der p 1 levels in food from the paper ($P = 0.02$) and plastic bags ($P = 0.03$) and house dust ($P = 0.015$). Carpets or rugs were significantly associated with Der p 1 levels in food stored in the paper ($P = 0.02$) and plastic bags ($P = 0.04$) and house dust ($P = 0.01$).

Acares[®] tests

The Acares[®] tests were negative in all the food samples at all the time points. The test results for the house dust samples on day 90 are shown in Table 2. There was a significant association with the Der p 1 levels in house dust at day 90 (logistic regression $P = 0.005$).

Levels of Der p 1

The Der p 1 levels in the food and house dust samples are shown in Figure 1 and Table 2. There was a significant effect of both time and type of storage throughout the study (two-way repeated-measures ANOVA, $P = 0.0023$ and $P = 0.0073$, respectively). However, time $\times$ storage interaction was significant ($P = 0.011$) and matching was not significant ($P = 0.29$), indicating that the changes in Der p 1 levels were not consistent between the types of storage or sample site at each time point.

Tukey's post hoc tests revealed that Der p 1 levels in food stored in the paper bags were significantly higher than those from the plastic bags and plastic boxes on days 30 ($P < 0.05$ and $P < 0.01$, respectively) and 60 ($P < 0.01$ and $P < 0.001$, respectively), and from the plastic boxes on day 90 ($P < 0.05$). The Der p 1 levels were higher in food stored in the paper bags on day 90 compared with days 0 ($P < 0.001$), 30 ($P < 0.001$) and 60 ($P < 0.01$). There were no significant differences in Der p 1 levels in food stored in the plastic bags or the plastic boxes at the different time points.

There was a significant difference between Der p 1 levels in food from the three storage types and house dust at each sample site on day 90 (Friedman tests, $P < 0.0001$). *Tukey's post hoc* tests revealed that Der p 1 levels were significantly higher in house dust than in food from the plastic bags ($P < 0.05$) and plastic boxes ($P < 0.01$) but not paper bags. There was a significant correlation between Der p 1 levels in house dust and Der p 1 levels in food from the paper bags and plastic bags (Pearson tests, $P < 0.0001$ and $P = 0.003$, respectively) but not the plastic boxes (Spearman rank test, $P = 0.40$).

Mite numbers

The numbers and identity of the mites found in each sample are shown in Figure 2 and Table 3. There was a significant effect of both time and type of storage (two-way repeated-measures ANOVA, $P < 0.0001$ and $P = 0.02$, respectively). There was no significant time $\times$ storage interaction ($P = 0.12$), and matching was effective ($P < 0.0001$), indicating that the differences in mite numbers were consistent between the types of storage and sample site at each time point. *Tukey's post hoc* tests revealed that there was a significant increase in mite

Table 1. Demographic data for each household

	Food storeroom	Floor in food storeroom	Carpet depth	Bedding in food storeroom	Vacuum cleaning	Filter vacuum cleaner	Insecticide in food storeroom	Animals in household	Dog breeds	Coat length	On-animal insecticide	No. people in household	Building type*	Building age (years)
1	Garage	Concrete and carpet	1–2 cm	Yes	Every other day	Yes	Indorex [®] January 2009	2 dogs 1 cat	German shepherd dog, rottweiler	Medium, short	Advantage [®] /Stronghold [®] every month	2	Detached 3 story	2
2	Kitchen	Wood laminate	n/a	No	Never	Yes	No	1 dog 1 cat	Collie cross	Medium	Frontline [®] every 3 months	3	Detached 3 story	35
3	Kitchen	Tiles	n/a	No	Weekly	No	Indorex [®] February 2009	1 dog	English springer spaniel	Medium	Frontline [®] every 2 months	3	Terrace 2 story	110
4	Kitchen	Tiles	n/a	Yes	Weekly	Yes	No	1 dog	Cairn terrier	Medium	Stronghold [®] every 1.5 months	2	Detached 2 story	20
5	Kitchen	Concrete and carpet	1 cm	No	Weekly	No	No	2 dogs	Newfoundland, greyhound	Long, short	No	5	Semi-detached 2 story	57
6	Hall	Tiles	n/a	No	3 times weekly	No	Indorex [®] December 2008	2 dogs	Pug, Staffordshire bull terrier	Short, short	Stronghold [®] every 3 months	2	Semi-detached 1 story	15
7	Kitchen	Carpet tiles	<1 cm	No	2–3 times weekly	Yes	No	2 dogs	Jack Russell terrier	Short	Frontline [®] every 2 months	2	Semi-detached 2 story	35
8	Living room	Laminate and rugs	1 cm	Yes	2 times weekly	Yes	No	2 dogs	Hungarian vizla	Short	Stronghold [®] /Frontline [®] every 3 months	2	Semi-detached 2 story	100
9	Kitchen	Wood	n/a	No	Never (washed every 7 days)	No	Indorex [®] August 2008	2 cats 1 dog	Border terrier	Short	Stronghold [®] every 3 months	2	Detached 2 story	30
10	Conservatory	Laminate	n/a	No	Weekly	No	No	1 dog 1 cat	English springer spaniel	Medium	Frontline [®] every 6 months	2	Semi-detached 2 story	7

n/a, not assessed. Drugs: Advantage[®] (imidacloprid, Bayer Animal Health, Newbury, UK); Frontline[®] (fipronil, Merial Animal Health, Harlow, UK); Indorex[®] (pyriproxifen/permethrin/piperonyl butoxide, Virbac Animal Health, Bury St Edmunds, UK); and Stronghold[®] (selamectin, Pfizer Animal Health, Sandwich, UK).

*All the buildings were brick built with gas central heating except site 2 (oil central heating).

Table 2. Der p 1 levels (in ng/g) and Acarex® test results in the food and house dust samples from day 0 to 90

Der p 1 (ng/g)												
Sample	Day 0	Day 30			Day 60			Day 90				Acarex® test on house dust at day 90
		Paper bag	Plastic bag	Box	Paper bag	Plastic bag	Box	Paper bag	Plastic bag	Box	House dust	
1	0.0	9.6	0.0	0.0	14.0	0.0	0.0	233.4	28.0	12.0	9895	Strongly positive
2	0.0	0.0	0.0	0.0	17.6	0.0	0.0	32.0	0.0	0.0	661.6	Slightly positive
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	549.3	Slightly positive
4	0.0	4.2	0.0	0.0	16.4	3.5	0.0	24.0	11.8	0.0	1917.8	Moderately positive
5	0.0	0.0	0.0	0.0	16.4	0.0	0.0	28.0	0.0	0.0	623.8	Slightly positive
6	0.0	2.4	0.6	0.0	10.6	7.0	1.8	16.8	16.8	8.4	1876.2	Slightly positive
7	1.0	0.0	0.0	0.0	7.0	0.0	0.0	6.4	0.0	0.0	539.8	Slightly positive
8	0.0	11.2	0.0	0.0	25.0	7.0	0.0	51.2	2.2	0.0	2942.6	Moderately positive
9	1.0	4.2	2.2	0.0	18.6	16.4	0.0	42.6	11.2	0.0	2594.2	Moderately positive
10	0.5	10.6	2.2	0.0	10.6	10.6	0.0	19.0	4.2	0.0	2671.2	Slightly positive
Mean	0.3	4.2	0.5	0.0	13.6	4.5	0.2	45.3	7.4	2.0	2427.2	–
SD	0.4	4.6	0.9	0.0	6.9	5.1	0.6	67.8	9.4	4.4	2791.7	–

All the Acarex® tests on the food samples were negative at all time points (data not shown). The house dust samples were analysed only at the day 90 time point.

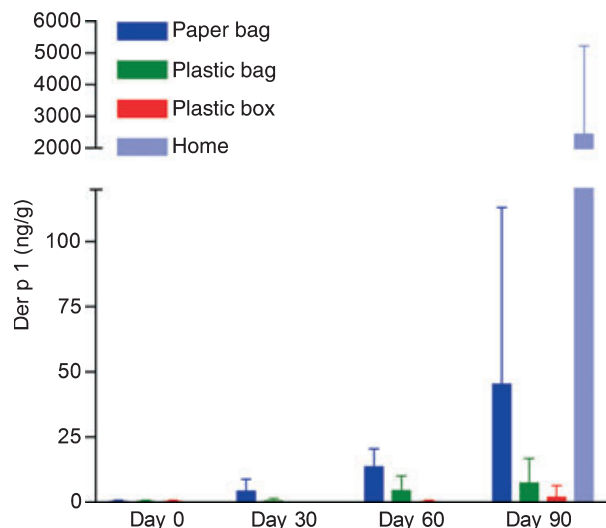


Figure 1. Levels of Der p 1 (in ng/g) in the food and house dust samples from day 0 to 90. The bars represent the means and SD. The house dust samples were only analysed at the day 90 time point.

numbers recovered from food stored in the paper bags on day 90 compared with day 0 ($P < 0.05$). There were otherwise no significant differences in mean mite counts in food from the different storage types at any time point.

There was a significant difference in the mite numbers seen in house dust and the food samples from each household on day 90 (Friedman test, $P < 0.0001$). *Dunn's post hoc* tests revealed that mite numbers in house dust were significantly higher than those in the food from the plastic bags ($P < 0.05$) and plastic boxes ($P < 0.001$), but not the paper bags. There was a significant correlation between the mite numbers in house dust and those in food from the paper bags (Pearson test, $P = 0.0007$), but not the plastic bags (Pearson test, $P = 0.07$) and the plastic boxes (Spearman rank test, $P = 0.63$).

Correlation with temperature and relative humidity

The temperature and relative humidity in each household are shown in Figure 3. There was a significant effect of

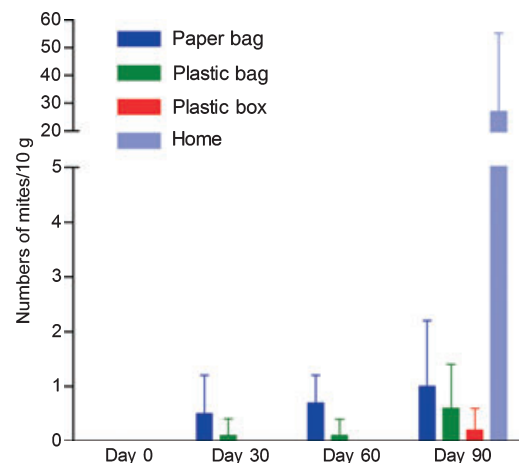


Figure 2. The numbers of mites observed in the food (10 g) and house dust samples (1 g) from day 0 to 90. The bars represent the means and SD. The house dust samples were only analysed at the day 90 time point. The mite numbers for the house dust samples are the number $\times 10$.

both time and sample site throughout the study (two-way repeated-measures ANOVA, $P < 0.0001$ in all cases), indicating that there was both a significant increase in temperature and relative humidity, and a significant difference between households. However, time $\times$ household interaction was also significant ($P < 0.0001$), indicating that the changes in temperature and relative humidity were not consistent between the sample sites at each time point.

There was no significant association of temperature and relative humidity with Der p 1 levels in house dust (multiple regression analysis, $P = 0.67$) on day 90, or the food from the paper bags ($P = 0.15$), plastic bags ($P = 0.08$) or plastic boxes ($P = 0.24$) from day 0 to 90. There was also no significant association of temperature and relative humidity with mite numbers in house dust (multiple regression analysis, $P = 0.65$) on day 90, or food from the paper bags ($P = 0.31$), plastic bags ($P = 0.59$) or plastic boxes ($P = 0.33$) from day 0 to 90.

Table 3. Mite numbers and identification (to genus) observed in the food (10 g) and house dust samples (1 g) from day 0 to 90

Site	1	2	3	4	5	6	7	8	9	10	Mean	SD
Day 0	0	0	0	0	0	0	0	0	0	0	0	0
Day 30												
Paper bag	1	0	0	1	0	0	0	1	0	1	0.4	0.5
	<i>Dermatophagoides</i> spp.			Unidentified				<i>Dermatophagoides</i> spp.	0	Unidentified		
Plastic bag	0	0	0	0	0	0	0	0	0	1	0.1	0.3
										<i>Dermatophagoides</i> spp.		
Box	0	0	0	0	0	0	0	0	0	0	0	0
Day 60												
Paper bag	2	0	0	1	0	0	0	1	0	1	0.5	0.7
	<i>Dermatophagoides</i> spp.			<i>Dermatophagoides</i> spp.				<i>Dermatophagoides</i> spp.		<i>Tyrophagus</i> spp.		
Plastic bag	1	0	0	0	0	0	0	0	0	1	0.2	0.4
	Unidentified									Unidentified		
Box	0	0	0	0	0	0	0	0	0	0	0	0
Day 90												
Paper bag	4	0	0	1	0	1	0	1	2	1	1.0	1.2
	<i>Dermatophagoides</i> spp. x2			<i>Dermatophagoides</i> spp.		<i>Dermatophagoides</i> spp.		<i>Dermatophagoides</i> spp.	Unidentified	<i>Tyrophagus</i> spp.		
Plastic bag	2	0	0	0	0	0	0	1	0	1	0.4	0.7
	<i>Dermatophagoides</i> spp.							Unidentified		Unidentified		
Box	1	0	0	0	0	0	0	0	0	0	0.1	0.3
	<i>Tyrophagus</i> spp.											
House dust	100	10	0	20	30	10	20	30	10	40	27	28.3
	<i>Dermatophagoides</i> spp. x7	Unidentified		<i>Dermatophagoides</i> spp.	<i>Dermatophagoides</i> spp. x2	<i>Dermatophagoides</i> spp.	Unidentified	<i>Dermatophagoides</i> spp.	Unidentified	<i>Dermatophagoides</i> spp. x2		
	Unidentified x3			Unidentified	Unidentified					Unidentified x2		

The total mite counts in the house dust samples are the count x10. The identity of the mites in the house dust samples is as seen in the 1 g sample.

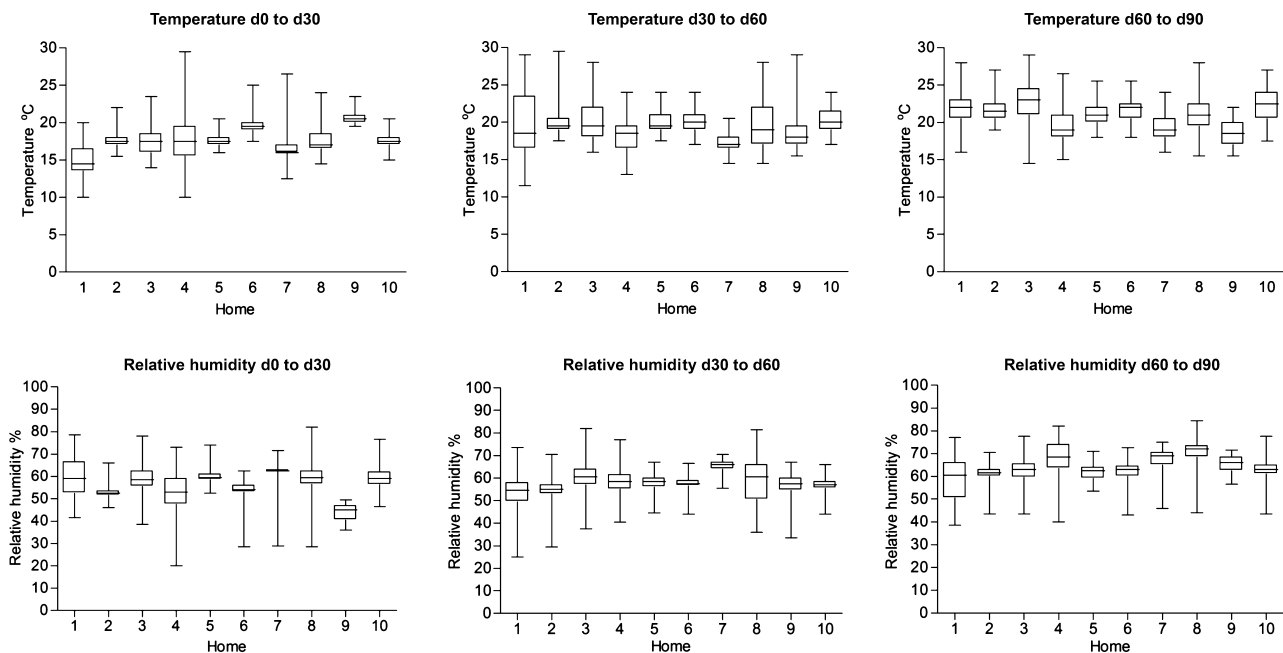


Figure 3. The temperature and relative humidity recorded in each household from day 0 to 90. The boxes illustrate the median, and 25% and 75% quartiles; the whiskers illustrate the maximal and minimal values.

Discussion

The Der p 1 levels and mite numbers increased throughout the study, although this was only significant for the food stored in the paper bags. The clinical significance of this, however, is unclear, as the Der p 1 levels and mite numbers were much lower than those in house dust recovered from the immediate environment of the stored food. It is therefore likely that the additional exposure to HDM and storage mite allergens in food is trivial compared with that in house dust. The accepted threshold for sensitization in humans is 2 µg Der p 1/g house dust, which was met or exceeded in six of 10 house dust samples. All of the food samples, in contrast, were less than one tenth of this level. Der p 1, however, is not a major allergen for dogs,^{3,39,40} and sensitization thresholds have not been established for the major *Dermatophagoides* allergens in cAD. Sensitization thresholds for storage mite allergens in dogs are also unknown, although the low mite numbers and negative Acarex[®] tests suggest that exposure was low in this study. It is possible that ingestion of allergen is a more potent route than inhalation or epicutaneous contact. Clinical lesions induced by oral exposure to *D. farinae* in experimentally sensitized beagles, however, were less marked and more transient than those induced by epicutaneous exposure.⁷ In contrast, environmental and oral exposure to *T. putrescentiae* were equally potent, although the small number of dogs and mixed ages in the study makes the significance of the data unclear.²⁹ The clinical importance of the route of exposure to storage mite allergens therefore needs further study.

The difference in mite contamination between the three types of food storage probably reflects their relative exposure to the environment. In an earlier study, no mites were found in food samples stored in a low dust mite

laboratory environment.³⁵ The Der p 1 levels and mite numbers in house dust in our study correlated with those in the paper and plastic bags, but not the boxes, suggesting that Der p 1 and mites accumulated in stored food from direct contact with a contaminated environment. Opening the bags and boxes was designed to mimic exposure to environment when opening for feeding. The paper bags did not otherwise provide an effective barrier; they could not be effectively closed, oils wicked through the paper and all were punctured. The oils and punctures could have facilitated ongoing entry of environmental allergens. Only one plastic bag was punctured, but the closures failed in four bags; three became blocked with food debris and one split from the side of the bag. The contents of the boxes, in contrast, were only exposed to the environment when opened. The authors of a recent study showing that *Tyrophagus* can survive on dry dog food speculated that food stored in a sealed container without the benefit of added moisture may not support mites.³¹ It is also possible that the correlation reflects similar environmental conditions favouring the survival and proliferation of house dust and storage mites at each site.^{31,32} Dry food, for example, will absorb moisture and spontaneously develop moulds that favour storage mite contamination and survival.³¹

On day 0 no mites were seen, only trace amounts of Der p 1 were detected, and the Acarex[®] tests were negative. This indicates that the food used in this study was not contaminated by HDM or storage mites at source, as reported elsewhere.^{33,34} A single type and batch of dry dog food was chosen in our study to minimize sources of variation between the samples. This does not preclude contamination of other types of food, as another study detected *A. siro* and an unidentified mite fragment in two of 10 different foods,³⁵ and storage mites can be found in pet food manufacturing plants.³⁰ Nevertheless, the only

study to date that reported heavy contamination of dry dog food concluded that environmental conditions were more important than type of food.³⁵

The temperature and relative humidity increased as the study progressed from spring to summer, although there was a significant difference between the households. This was not, however, correlated with Der p 1 levels or mite numbers in the food samples or house dust. Indoor temperature and relative humidity have been correlated with HDM populations, but differences would not be expected within the range seen in this study.⁴¹ In most cases, the temperature ranged from 15 to 25 °C and the relative humidity between 50% and 70%, which permits the survival and proliferation of *D. pteronyssinus*, *Tyrophagus* and *Acarus*.^{13,24,42,43} The optimal humidity for storage mites, however, is generally higher (>75%) than for HDM (~60–70%).^{20,23,24,29} This suggests that HDM may have been favoured in the households in this study, and that food storerooms commonly thought to favour storage mites may be equally, if not more, conducive to HDM proliferation. The temperature and relative humidity were lower throughout most of the trial compared with those reported in the study of food stored in Spain.³⁵ That trial reported greater mite contamination than in our study and others performed in temperate climates.^{33,34} In contrast, there was no contamination of food samples stored in a laboratory at 16 ± 2.3 °C and 68 ± 7.3% relative humidity,³⁵ conditions similar to those in our households for most of the study. A drawback of our study, however, was that the temperature and relative humidity were only recorded in the food storeroom. It is possible that the microclimate differed in the food bags and boxes, and that this may have affected mite survival and proliferation at each location.

It proved impossible to sample the house dust consistently at each site at each time point, so correlations were only possible for day 90. Levels of Der p 1 are very stable and unlikely to change in the absence of management changes,⁴⁴ but it is possible that there may have been short-term fluctuations in mite numbers. It is also possible that differences between households were insufficient to affect house dust and storage mite populations. A previous study of Der p 1 levels in the same region also found few significant factors affecting allergen levels in different households.¹¹ Nevertheless, the presence of carpets and bedding in the food storeroom was related to increased numbers of mites and Der p 1. Older carpets are known to harbour more mites,^{11–13,24} although we did not record this in our study. Soft furnishings are known to provide ideal substrates for house dust mites,^{11,20} although this was contradicted in one recent US study.⁴¹ Coat length and on-animal ectoparasiticide use have previously been shown not to affect Der p 1 levels in dust from carpets, bedding and coats.¹² Use of environmental insecticides decreased Der p 1 levels in an earlier study,¹¹ but there was no effect in this study. The impact of insecticides, however, was only significant in living rooms, which were not sampled here (except for site 8). The aim of this study was to evaluate house dust and storage mite contamination of dog food in household conditions. A variety of dog-owning households were chosen from a convenience population to reflect normal household envi-

ronments, and use and storage of dog food. The number of homes was limited to 10 by logistic and other resources, and the low statistical power probably affected our ability to identify household factors associated with the degree of mite contamination at each site. It would therefore be interesting to repeat this study in households controlled for specific factors, such as high or low mite numbers, age, type of food storeroom etc.

Previous studies have reported that mite detection is less sensitive than *in vitro* assays for mite allergens.^{12,13,35} In one study, *D. pteronyssinus* was only found in 11.8% of samples, whereas 60.8% were positive for Der p 1.¹² A further 7.4% of samples were positive for storage mites, but this may underestimate the population. Specific ELISAs for *Acarus* and *Tyrophagus* have been evaluated in stored cereals,⁴⁵ but there are no currently available reagents to detect storage mite allergens. Detection of guanine levels using Acarex® tests can provide an indication of mite populations in stored foods,^{35,46} although this does not differentiate between storage and house dust mites and other Arachnidae. In our study, all the house dust samples were positive, and the results were significantly associated with Der p 1 levels. In contrast to an earlier report,³⁵ however, all the Acarex® tests on the food samples were negative, suggesting that mite-derived guanine levels were below the threshold for detection. BT® traps (Central Science Laboratory, York, UK) incorporating a lure for storage mites have been used to evaluate contamination of stored human and animal foodstuffs.^{30,35} They were not used in our study because they require several days of exposure to food, have been shown to be less sensitive than flotation studies³⁵ and were not available in sufficient numbers. There is no standardized flotation method for evaluating mite numbers.^{12,35,45,47} Prior evaluation of a number of techniques showed that the one used in our study was sensitive and reproducible (data not shown).

Dermatophagoides spp. mites were seen in both house dust and food samples, whereas *Tyrophagus* spp. mites were seen only in the food samples. This is consistent with most reports,^{2,20} although not with an early British study that identified both house dust and storage mites in house dust.⁴⁸ Many mites, however, were fragmented and/or desiccated, making identification difficult. The high proportion of unidentified mites in both our house dust and food samples means that it is difficult to draw firm conclusions about the relative distribution of species among house dust and food.

In conclusion, this study has shown that house dust and storage mites and their allergens can contaminate dry dog food stored in household conditions. There is, however, little evidence that this is a clinically significant source of exposure for atopic dogs in temperate maritime climates. Nevertheless, storing food in sealed containers for no more than 3 months and avoiding bedding or carpets/rugs in the food storeroom would appear useful to minimize contamination.

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Résumé Les aliments secs pour animaux de compagnie sont une source potentielle d'exposition aux allergènes d'acariens de poussière de maison et d'acariens de stockage dans le cadre de la dermatite atopique canine (DAC). Cette étude évalue la contamination de la poussière de maison et des croquettes stockées dans des sacs en papiers, des sacs et des boîtes en plastique refermables dans 10 habitations pendant 90 jours à l'aide d'Acarex-test[®], un test ELISA Der p 1 et un test de flottaison d'acariens. Les Acarex-tests[®] étaient négatifs pour tous les échantillons d'aliments mais positifs pour tous les échantillons de poussière de maison. Les niveaux de Der p 1 et le nombre d'acariens étaient significativement plus élevés pour les aliments conservés dans des sacs en papier ($P = 0.0073$ et $P = 0.02$), mais pas pour ceux conservés dans les sacs ou les boîtes en plastique. Le nombre d'acariens et les niveaux de Der p1 étaient 10 à 1000 fois plus élevés dans la poussière de maison que dans les échantillons d'aliments correspondants ($P < 0.0001$). Il y avait une corrélation significative entre Der p1 dans la poussière de maison et la nourriture stockée dans des sacs en papier ($P < 0.0001$) et des sacs en plastique ($P = 0.003$), ainsi qu'entre les nombres d'acariens de la poussière de maison et la nourriture conservée dans des sacs en papier ($P = 0.0007$). La literie et les tapis étaient significativement associés avec Der p 1 dans la poussière de maison ($P = 0.015$ et $P = 0.01$), l'alimentation conservée dans le papier ($P = 0.02$) ainsi que dans les sacs en plastique ($P = 0.03$ et $P = 0.04$). Les acariens ont été identifiés dans 6/10 sacs en papier, 3/10 sacs en plastique, 1/10 boîtes en plastique et 9/10 prélèvements de poussière de maison. Ils comprenaient *Dermatophagoides* (54%), *Tyrophagus* (10%; tous à partir de l'alimentation) et des acariens non identifiés (36%). La conservation de l'alimentation dans des boîtes en plastique refermables a largement prévenu leur contamination pendant trois mois. L'exposition aux acariens et aux protéines d'acariens dans l'alimentation stockée apparaît, cependant, insignifiante en comparaison avec la poussière de maison.

Resumen La comida seca para animales domésticos es una fuente de exposición a alérgenos de ácaros del polvo y de almacén en dermatitis atópica canina (cAD). Este estudio evaluó la contaminación del polvo del hogar y comida seca de perro almacenada en bolsas de papel, bolsas de plástico y cajas de plástico con tapas en diez hogares durante 90 días utilizando la prueba Acarex[®] para guanina, una prueba de ELISA con Der p1 y flotación de los ácaros. Las pruebas de Acarex[®] fueron negativas en todas las muestras de comida, pero positivas en todas las muestras de polvo. Los niveles de Der p1 y el número de ácaros aumentó de forma significativa en la comida de bolsas de papel ($P = 0.0073$ y $P = 0.02$), pero no en las bolsas de plástico ni en las cajas. El número de ácaros y los niveles de Der p1 fueron 10-1000x mayores en el polvo del hogar que en las correspondientes muestras de comida ($P < 0.0001$). Hubo una correlación significativa entre Der p1 en el polvo del hogar y la comida de las bolsas de papel ($P < 0.0001$) y de plástico ($P = 0.003$, y el número de ácaros en el polvo del hogar y la comida de las bolsas de papel ($P = 0.0007$). Las camas y alfombras estaban significativamente asociadas con los niveles de Der p1 en el polvo del hogar ($P = 0.015$ y $P = 0.01$) y la comida de las bolsas de papel ($P = 0.02$) y de plástico ($P = 0.03$ y $P = 0.04$). Los ácaros se identificaron en 6/10 bolsas de papel, 3/10 bolsas de plástico, 1/10 cajas de plástico y 9/10 muestras de polvo. Estos englobaron *Dermatophagoides* (54%), *Tyrophagus* (10%; todos de comida) y ácaros no identificados (36%). El almacenamiento de comida en cajas de plástico con tapa evitaba en gran parte la contaminación durante tres meses. La exposición a ácaros y proteínas de los ácaros en toda la comida almacenada fue sin embargo mínima en comparación con la de polvo del hogar.

Zusammenfassung Trockenes Hundefutter ist eine mögliche Quelle für eine Belastung durch Hausstaub- und Futtermittelmilbenallergene bei caniner atopischer Dermatitis (cAD). Diese Studie evaluierte die Kontaminierung von Hausstaub und Trockenfutter, welches in zehn Haushalten 90 Tage lang in Papiersäcken, in verschleißbaren Plastiksäcken und in verschleißbaren Plastikboxen aufbewahrt wurde. Es wurden Acarex[®] Tests für Guanin, sowie ein ELISA für Der p 1 und eine Milben Flotation verwendet. Während die Acarex[®] Tests bei allen Futterproben negativ waren, waren sie bei allen Hausstaubproben positiv. Die Der p 1 Gehalte und die Anzahl der Milben waren in Futter aus Papiersäcken signifikant erhöht ($P = 0.0073$ und $P = 0.02$), während dies bei Proben aus den Plastiksäcken bzw. -boxen nicht der Fall war. Die Anzahl der Milben und der Gehalt an Der p 1 waren im Hausstaub 10-1000x höher als in den entsprechenden Futterproben ($P < 0.0001$). Es bestand eine signifikante Korrelation zwischen Der p 1 im Hausstaub und im Futter aus Papiersäcken ($P < 0.0001$) und aus Plastiksäcken ($P < 0.003$), sowie zwischen der Anzahl der

Milben im Hausstaub und im Futter aus Papiersäcken ($P = 0,0007$). Hundebetten und Teppiche zeigten einen signifikanten Zusammenhang zwischen den Der p 1 Werten im Hausstaub ($P = 0,015$ und $P = 0,01$), sowie im Futter aus Papier- ($P = 0,02$) und aus Plastiksäcken ($P = 0,03$ und $P = 0,04$). Es wurden in 6/10 Papiersäcken, in 3/10 Plastiksäcken, in 1/10 Plastikboxen und in 9/10 Hausstaubproben Milben identifiziert. Diese waren repräsentiert durch *Dermatophagoides* (54%), *Tyrophagus* (10%; alle aus Futter) und nicht identifizierte Milben (36%). Die Aufbewahrung von Futter in verschließbaren Plastikboxen verhinderte weitgehend eine Kontaminierung für eine Dauer von drei Monaten. Allerdings erschien die Belastung durch Milben und Milbenproteine in allen Aufbewahrungsmedien im Vergleich zu Hausstaub unbedeutend.

要約 ドライフードは犬アトピー性皮膚炎 (cAD) において、チリダニや貯蔵ダニのアレルゲンへの暴露源になる可能性がある。この研究は紙袋、密封可能なビニール袋、密封可能なプラスチック容器に保存したハウスダストとドライフード中の汚染を、グアニンを検出する Acares®テスト、Der P1 の ELISA、およびダニ浮遊法で評価した。Acares®テストはハウスダスト以外のすべてのフードサンプルで陰性であった。DerP1 の値とダニ数は、紙袋に入っていたフードで優位に増加した ($p=0.0073$ and $p=0.02$) が、ビニール袋やプラスチック容器のものでは差はなかった。ダニ数と Der P1 値は対応するフードのサンプルと比較してハウスダストでは 10-1000 倍高かった ($P < 0.0001$)。紙袋 ($P < 0.0001$) とビニール袋 ($P = 0.0007$) に入っていたフードとハウスダスト中の Der p 1 の間には有意な差があり、紙袋中のフードとハウスダスト中のダニの数の間にも有意な差が見られた。寝具や敷物とハウスダスト中 ($P = 0.015$ と $P = 0.01$)、紙袋のフード中 ($P = 0.02$)、ビニール袋中 ($P = 0.03$ と $P = 0.04$) の Der p 1 レベルは有意に相関していた。ダニは 6/10 の紙袋、3/10 のビニール袋、1/10 プラスチック容器と 9/10 のハウスダストから検出された。検出されたダニは *Dermatophagoides* (54%), *Tyrophagus* (10%; すべてがフードから検出) と同定できないダニ (36%) であった。密封可能なプラスチック容器に保存されたフードは概ね 3 カ月間は汚染を免れた。保存された全てのフードからのダニとダニタンパクに対する暴露は、しかしながら、ハウスダストと比較すると極めて少ないことが明らかになった。