

Commercial dry dog food in the north central United States is not contaminated by *Dermatophagoides* house dust mites

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Abstract Contamination of home-stored cereal grain food products with *Dermatophagoides* spp. house dust mites (HDM) was reported recently, along with anaphylaxis after consumption of these foods by dust mite-allergic people. We hypothesized that commercial dry dog food could become similarly contaminated, particularly if stored improperly, and could eventually contribute to allergic signs in dogs. Newly purchased bags of dry dog food ($n = 30$), from a variety of sources and manufacturers, and client samples of dry dog food ($n = 50$), stored under a variety of conditions, were obtained. Food samples were extracted in aqueous buffer, and extracts were assayed using ELISA for *Dermatophagoides* group II (Der II) allergen, as a marker for the presence of HDM. Der II allergen was not detected in any of the 30 newly purchased or 50 stored samples tested. Positive control samples consisting of house dust or dog food mixed with house dust, similarly extracted, and *Dermatophagoides* commercial allergen extract were positive for Der II in the same assay. We could find no evidence of HDM contamination in newly purchased or stored commercial dry dog food in the north central United States.

Keywords: dog, *Dermatophagoides*, food, mite.

INTRODUCTION

House dust mites (HDM) of the genus *Dermatophagoides* are frequently implicated in hypersensitivity reactions occurring in both domestic animals and human beings. Although the major route of exposure to HDM allergens is thought to be via inhalation, several reports have documented severe allergic reactions in people following ingestion of foodstuffs contaminated with HDM.^{1,2,3} Recent studies have determined that many cereal grain products intended for human consumption, such as flours and breakfast cereals, can become contaminated with *Dermatophagoides* mites after purchase and during storage in the household. The rate of contamination varies with storage conditions, with tightly sealed containers and refrigeration generally preventing mite contamination, and unsealed storage at room temperature encouraging it.⁴

Because grain-based dry pet foods are typically purchased and stored under similar conditions in the household, these findings raise the possibility that pet food also could become contaminated. Indeed, when *Dermatophagoides* mites are raised in the laboratory, dry dog food fully supports their growth and reproduction, and is an excellent nutrient medium on which to rear them.⁵ HDM-contaminated dog food could, therefore, represent a source of dust mite allergen when

ingested by pets. Such exposure could serve either to sensitize an animal initially, or to exacerbate allergic signs in a hypersensitive pet.

The objective of this study was to survey a wide variety of commercial dry dog foods, both newly purchased and stored under various conditions in client households, for evidence of contamination by *Dermatophagoides* mites. As a marker for mite contamination during manufacture or storage, we assayed the foods for *Dermatophagoides* group II allergen, a heat-stable antigenic component common to both *D. farinae* and *D. pteronyssinus*.⁶

MATERIALS AND METHODS

Sample collection

A total of 30 bags of commercial dry dog food was purchased at six different retail locations in south central Wisconsin, USA, in June and July. The 30 samples consisted of an equal mixture of food types, designated as premium (more expensive brands sold only at pet stores and veterinary clinics), standard (nationally distributed brands available at supermarkets) or generic (low-cost brands typically sold under a store's private label).

Fifty samples of stored dry dog food were solicited from owners of patients presented to the Veterinary Medical Teaching Hospital. Each owner was instructed to mix the contents of their current bag of dog food as stored at home, then withdraw a sample into a self-sealing plastic bag. Owners completed a

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questionnaire specifying the brand and type of food, approximate dates the bag was purchased and opened, and storage conditions (inside the house vs. outdoors including garage; unsealed, loosely sealed or tightly sealed bag). The client-provided samples were collected between March and July, i.e. corresponding to food storage during both periods of warm and cool weather. Both purchased and client-provided samples were immediately stored at -20°C , to prevent further proliferation of any mites present, then extracted within 2 months of frozen storage.

As positive controls, samples of house dust were obtained from three separate household vacuum cleaners in current use (June), stored at -20°C and further processed within 1 month of collection.

Sample extracts

Samples were extracted in aqueous buffer in accordance with published techniques.^{6,7} Food samples were ground individually in a household coffee grinder (model KSM2B, Braun Inc., Woburn, MA), then sifted through a 355- μm wire mesh screen (W.S. Tyler, Mentor, OH). The size of the screen permitted the passage of any whole mites while retaining larger debris. Weighed aliquots (100 mg) of sifted food were added to tubes containing 2 mL of borate-buffered saline-Tween solution (0.05 M sodium borate in 0.15 M NaCl, pH 8.0, with 0.1% v/v Tween-80) and shaken vigorously for 2 h at 25°C . Tubes were centrifuged for 20 min at 1200 g to pellet food debris. The supernatant was recovered and stored at -20°C , as specified by the manufacturer of the Der II ELISA kit used (package insert, *Dermatophagoides* group II allergen ELISA kit, Indoor Biotechnologies, Inc., Charlottesville, VA), then assayed for mite allergen within 4 weeks of storage. The three frozen-stored house dust samples, and a sample of frozen-stored dry dog food in mixed with the house dust, were similarly extracted. Frozen extracts were thawed and refrozen no more than twice in the course of the assays.

ELISA for Dermatophagoides group II allergen

Samples were assayed for *Dermatophagoides* group II (Der II) content with a two-site sandwich ELISA method, using commercially available monoclonal antibodies against Der II and a commercial Der II reference standard (Indoor Biotechnologies).⁷ Briefly, 96-well polystyrene microtitre plates (Corning Costar, Corning, NY) were coated with 100 μL capture antibody 1D8 at 1 μg per well in 0.1 M carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C . Wells were washed six times with 0.15 M NaCl containing 0.05% v/v Tween-20, then blocked with 0.4% bovine serum albumin solution for 1 h at 25°C . Plates were again washed, then 100 μL of samples, standards and control extracts were added. Samples and reagents were diluted in 0.05 M Tris-buffered 0.15 M NaCl, pH 7.4, containing 0.1% Tween-20 and 0.1% bovine serum albumin. Food and dust sample extracts were thawed, mixed by vortexing and added undiluted and at three

additional twofold serial dilutions (1/2 to 1/8). Standard Der II extract was added at twofold serial dilutions from 250 to 0.5 ng mL⁻¹. A negative control (buffer only) was included on each plate. Commercially produced HDM allergen extract (Greer Laboratories, Lenoir, NC) stored at 4°C was used as a positive control sample on each plate to confirm assay performance. Plates were incubated for 1 h at 25°C , washed, then incubated for an additional 1 h with 100 μL detection antibody (biotin-conjugated antibody 7A1, 1/3000 dilution). After washing, plates were incubated for 30 min with 100 μL of 1/5000 streptavidin-peroxidase (Sigma, St. Louis, MO). Following a final washing, ABTS substrate solution (Sigma) was added, and colour development was monitored. Plates were read on a microplate reader (Bio-Tek, Winooski, VT) at 405 nm when the highest standard well reached an optical density between 1.2 and 1.6. A standard curve for optical density vs. Der II concentration was prepared from the standard well data, and the concentration of Der II (ng mL⁻¹) in sample extract wells was calculated using the microplate reader software (KineticCalc). Der II concentration in the original food samples was calculated and expressed as nanograms of Der II per gram dry weight of food (ng g⁻¹).

Effect of storage conditions on Der II assay

A set of experiments was performed to assess any possible effect of frozen storage of food samples or extracts in altering Der II concentrations measured in the ELISA. These experiments duplicated the most severe storage and handling conditions to which the foods and extracts were exposed over the course of the study, i.e. storage of food at -20°C for up to 2 months, storage of extract at -20°C for up to 1 month, and freeze-thawing of extract up to two times. Six food samples were infested experimentally with live cultures of *Dermatophagoides* mites (a gift from Heska Corporation). After 2–3 weeks, well-mixed portions of the foods were aliquoted. One aliquot was freshly extracted using the above procedure (designated extracts F). A portion of each F extract was thawed at room temperature then refrozen, twice (extracts FT). Extracts F and FT were then assayed for Der II in parallel on the same day. An additional portion of extracts F were stored at -20°C for 4 weeks (extracts FS), and the FS extracts were then assayed for Der II content. Another aliquot of each infested food was stored at -20°C for 2 months, then extracted (extracts S) and assayed for Der II content. Comparisons made with the frozen extracts were expressed as a per cent recovery of the Der II content assayed in fresh (F) samples.

RESULTS

Food samples

Client-provided food samples included 37 premium-brand, 11 standard-brand and two generic-brand foods. Storage conditions of the foods are shown

Table 1. Storage conditions of client-provided dry dog food samples. Outdoor samples included those stored in garages

Storage condition	Number of samples
Indoors, tightly sealed	20
Indoors, loosely sealed	18
Indoors, unsealed	7
Outdoors, tightly sealed	3
Outdoors, loosely sealed	2
Outdoors, unsealed	0

(Table 1). The mean duration the food had been stored under the given conditions was 28.5 days (range, 2–114 days).

Assay for Der II allergen

Observation of the standard curve data indicated that optical densities consistently above background were obtained down to the 2 ng mL⁻¹ standard. A minimum detectable Der II concentration of 2 ng mL⁻¹ corresponded to 40 ng Der II per gram of original dry sample (40 ng g⁻¹). *Dermatophagoides* group II allergen was not detected at this level in any of the purchased or client-provided dry dog food samples. The allergen was detected in the three similarly extracted house dust samples (220, 120 and 140 ng g⁻¹) and in the sample of dog food mixed with dust (65 ng g⁻¹). The commercial HDM extract used as a positive control was determined to contain $\approx$ 56 000 ng mL⁻¹ of Der II allergen.

Comparison of assay results for fresh vs. frozen extracts revealed a mean recovery of 98.5% of Der II assayed in FT vs. F samples. The FT samples assayed were sometimes higher and sometimes lower than the F samples, suggesting that this variation was related to inherent assay variability and not to degradation with freeze–thawing. Comparing extract series F with extract series FS and S similarly, mean Der II recoveries were of 135 and 92%, respectively. The latter comparisons may have showed more variability in part because the assays were carried out on different plates and at different times rather than on the same plate on the same day; and the S extracts were an entirely new extract prepared from a frozen food aliquot, and thus were additionally subject to the variation related to extraction procedures. Thus, we found no evidence that freeze–thawing or frozen storage at –20 °C for up to 2 months resulted in any consistent reduction in detectable Der II allergen.

DISCUSSION

The results of this study suggest that contamination of commercial dry dog food with *Dermatophagoides* HDM, either during its manufacture or during storage after purchase, occurs rarely or not at all, at least in the geographical region sampled.

Evidence for contamination of foods with HDM can be via either direct microscopic identification of mites, or detection of one or more specific mite components

in the food. The latter method has advantages over direct microscopy, because it detects ng quantities of protein originating from mite fragments or faecal pellets, which may be present even though no whole mites are seen. *Dermatophagoides* group II allergens are 14 kDa proteins which, along with the group I allergens, are the most common cause of human sensitization to HDM.⁸ We chose to assay Der II allergen because it is relatively stable to heat, pH changes and enzymatic digestion (which may occur during food processing), is common to both *D. farinae* and *D. pteronyssinus* species, and is distinct from storage mite allergens.^{8,9} Although the other major HDM allergen (Der I) also is detectable in even larger amounts in mite-contaminated foods, it is mite species-specific, more labile and its detection can be complicated by binding with cereal prolamins present in some flours.^{8,10}

It is possible that HDM allergens were present in the food samples, but at levels below the sensitivity of the Der II ELISA assay. However, concentrations of Der II reported in HDM-contaminated foods for human consumption generally range from 550 to 36 000 ng g⁻¹, which is well within the range of detection in our assay.³ In addition, two prior studies provide evidence that concentrations of mite allergen below 40 ng g⁻¹ may not be clinically important: first, in a large longitudinal study of long-term exposure of children to mite allergen, the concentrations of Der I allergen associated with sensitization were > 2000 ng g⁻¹ house dust.¹¹ Second, low, but detectable, levels of Der f 1 allergen ($\approx$ 125 ng g⁻¹) can be found in some pancake flours as purchased, but the individual boxes of flour responsible for human anaphylactic reactions contain the allergen at a concentration 100–1000 times higher.¹² Thus, clinical sensitization and clinical signs associated with mite-contaminated foods occur at mite allergen concentrations logarithmically higher than the detection limit of the Der II assay.

We found no evidence that freeze–thawing or short-term (1–2 months) storage of dog food or food extracts at –20 °C had a consistent or detrimental effect on measured Der II concentrations in the ELISA. Variable reduction of 25–50% in assayed Der II content of mite extracts stored at –20 °C has been reported recently.¹³ This report tested different extracts to those in our study (glycerin-containing commercial house dust mite allergen extracts) and a longer storage period (6 months), which may explain the difference in results. Nevertheless, it would be prudent in future studies to assay the extracts as soon as possible after extraction to minimize possibility of degradation during storage.

The lack of evidence for contamination of dry dog food by *Dermatophagoides* mites does not preclude contamination by other pyroglyphid mites or with storage mites; the latter also are an important cause of occupational allergy in man and may sensitize dogs.^{2,14} In addition, many reports of HDM contamination of human foodstuffs originate from subtropical climate regions,^{1–4} rather than the relatively cold and dry north central United States; it is possible that HDM

contamination of dog food may occur more readily in warmer geographical regions.

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Résumé Il a récemment été décrit une contamination de certains aliments composés de céréales par des acariens des poussières *Dermatophagoides* spp. (HDM). En outre, certains malades allergiques aux acariens des poussières ont présenté un choc anaphylactique après l'ingestion de ce type d'aliments. Nous avons supposé que la nourriture sèche industrielle du chien pouvait être également contaminée, notamment en cas de mauvais stockage, et pourrait ainsi contribuer aux signes cliniques chez les chiens allergiques. Des sacs d'aliments neufs ($n = 30$), de sources et de marques variées, et des prélèvements obtenus à partir de sacs de clients ($n = 50$), stockés dans des conditions diverses, ont été examinés. Des extraits d'aliments ont été obtenus et analysés avec un test ELISA pour vérifier la présence d'allergènes *Der* II (*Dermatophagoides* group II), considéré comme un marqueur des HDM. Aucun allergène *Der* II n'a été détecté, ni dans les 30 sacs neufs, ni dans les 50 prélèvements à partir de nourriture stockée. En revanche, en utilisant de la poussière de maison, des aliments mélangés avec de la poussière, ou des extraits commerciaux d'allergènes de *Dermatophagoides*, les mêmes tests ont été positifs. En conclusion, aucune contamination par des HDM n'a été observée dans cette étude portant sur des aliments industriels secs du commerce. [DeBoer, D. J., Schreiner, T. A. *Commercial dry dog food in the north central United States is not contaminated by Dermatophagoides house dust mites.* (L'alimentation industrielle sèche pour chiens n'est pas contaminée par les acariens des poussières *Dermatophagoides*, dans le centre et le Nord des USA.) *Veterinary Dermatology* **12**: 183–187.]

Resumen Se describió recientemente la contaminación de alimentos basados en granos de cereales y almacenados en casa, con ácaros del polvo *Dermatophagoides* spp. (AP), junto con anafilaxia, después del consumo de estos alimentos, en personas alérgicas al ácaro del polvo. Nuestra hipótesis es que los alimentos comerciales secos para perros podrían contaminarse de forma similar, especialmente si se almacenan de forma inadecuada, y podrían eventualmente contribuir a los síntomas de alergia en los perros. Se obtuvieron bolsas de comida seca para perros de adquisición reciente ($n = 30$), de varios orígenes y fábricas, y muestras de alimentos secos de clientes ($n = 50$), almacenados en diferentes condiciones. Se extrajeron muestras de alimentos en tampón acuoso, y los extractos fueron probados utilizando ELISA para alérgeno de *Dermatophagoides* grupo II (Der II), como marcador de la presencia de AP. No se detectó alérgeno Der II en ninguna de las 30 muestras de adquisición reciente, ni en las 50 muestras almacenadas. Las muestras control positivas, consistentes en polvo doméstico o comida de perros mezclada con polvo doméstico, extraídas de manera similar, y extracto comercial de alérgeno de *Dermatophagoides* fueron positivos a Der II en la misma prueba. No pudimos encontrar ninguna prueba de contaminación por AP en alimentos comprados recientemente ni en alimentos secos comerciales almacenados en el centro-norte de los Estados Unidos. [Deboer, D. J., Schreiner, T. A. *Commercial dry dog food in the north central United States is not contaminated by Dermatophagoides house dust mites.* (La comida comercial seca de perro en la zona centro-norte de los Estados Unidos no se encuentra contaminada por ácaros del polvo doméstico *Dermatophagoides*.) *Veterinary Dermatology* **12**: 183–187.]

Zusammenfassung Kontaminierung der im Haus gelagerten, getreidehaltigen-Nahrungsmittel mit Hausstaubmilben *Dermatophagoides* spp. (HSM) wurde vor kurzem zusammen mit Anaphylaxis nach Verbrauch dieser Nahrungsmittel durch Milben-allergische Menschen berichtet. Wir stellten die Hypothese auf, dass kommerzielle Hundetrockenfutter besonders bei unsachgemäßer Lagerung ähnlich kontaminiert werden und zur allergischen Symptomatik bei Hunden beitragen könnten. Von mehreren Verkaufsstellen und Herstellern frisch gekaufte Hundetrockenfutterbeutel ($n = 30$) und Proben von unter variierenden Bedingungen gelagerten Hundetrockenfuttern von Kunden ($n = 50$) wurden erworben. Futtermittelproben wurden in wässriger Pufferlösung extrahiert, und Extrakte wurden mittels ELISA auf das *Dermatophagoides* Gruppe II Allergen (Der II) als Marker für das Vorhandensein von Hausstaubmilben geprüft. Der II Allergen wurde in keiner der Proben gefunden. Die ähnlich extrahierten positiven Kontrollproben, die aus Hausstaub oder mit Hausstaub kontaminiertem Hundefuttern bestanden, und kommerzieller *Dermatophagoides*-Allergenextrakt testeten positiv. Wir konnten keinen Hinweis auf HSM-Kontamination in gekaufter oder gelagerter kommerzieller trockener Hundenahrung im Norden des Zentrums der Vereinigten Staaten finden. [Deboer, D. J., Schreiner, T. A. *Commercial dry dog food in the north central United States is not contaminated by Dermatophagoides house dust mites.* (Kommerzielles Hundetrockenfutter im Norden der zentralen Vereinigten Staaten ist nicht durch *Dermatophagoides* Hausstaubmilben kontaminiert.) *Veterinary Dermatology* **12**: 183–187.]