

Evaluation of storage mite contamination of commercial dry dog food

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What is known about the topic of this paper

- Storage mites are potentially important allergens in canine atopic dermatitis.
- Dry pet food has been suggested as a potential source of storage mite exposure for dogs.
- There are limited studies on the occurrence of storage mites in dry pet food.

What this paper adds to the field of veterinary dermatology

- Commercial dry dog diets may contain storage mites.
- Dry dog food can become contaminated by storage mites after being opened and stored under specific environmental conditions.

Abstract

Storage mites may be considered important allergens in dogs with atopic dermatitis. High sensitization rates to *Tyrophagus*, *Acarus*, and *Lepidoglyphus* species have been reported in atopic dogs, and dry pet food has been suggested as a potential source of storage mite exposure. The aim of the present study was to evaluate commercial dry dog food for contamination with storage mites, and how storage time and conditions could influence the risk of contamination. Ten different premium commercial dry dog foods formulated for skin disorders were selected. Food bags were opened and stored for 6 weeks under two different environmental conditions. At different time points, samples from each bag were collected and analysed by microscopy, guanine test, storage mite-specific traps, and a modified flotation technique.

On opening, two storage mites identified as *Acarus siro* were isolated from one of the 10 bags by flotation technique, indicating that storage mites can be present in packaged dry dog food bags. After 5 weeks of storage under environmental conditions optimal for mite growth (23.2 ± 2.1 °C and $71 \pm 5.6\%$ of relative

humidity), mites were detected by microscopic observation in nine of the 10 diets. When mites were identified by the flotation technique, *Tyrophagus* spp. were found to be the most common contaminating species. These results show that dry dog food can be a suitable substrate for storage mite reproduction, and that environmental and storage conditions may influence food contamination and mite development.

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Conflict of Interest

No conflict of interest has been declared.

Introduction

Storage or forage mites belonging to the Glycyphagidae and Acaridae families including *Tyrophagus*, *Acarus*, *Lepidoglyphus* and *Glyciphagus* are considered to be a major source of environmental allergens for dogs with atopic dermatitis.^{1–3} These mites are often found in stored hay, straw, grain, and dry feed stuff.^{4–6} Together with house dust mites, storage mite allergens are considered one of the main causes of human allergy, causing respiratory symptoms after occupational exposure in farms, grain stores and bakeries.^{7,8} Moreover, several reports have documented severe allergic reactions in human patients following ingestion of foodstuffs contaminated with mites.^{9–11}

Sensitivity to different forage mite species alone or in combination with house dust mites has been demonstrated by intradermal test in a group of atopic dogs.¹² In addition, an extremely high prevalence of serum IgE against storage mites has been found in atopic dogs.⁶ These results may suggest the presence of cross-reactions between storage mites and other mite species (*Dermatophagoides* sp.), but could also indicate the presence of storage mites in the environment of the dog (house dust, bedding dust), as demonstrated by some studies.^{13,14} Recently, studies carried out in a dry pet food factory in the UK showed that processing and packing areas can be contaminated by *Tyrophagus* spp. and *Acarus siro*; *T. putrescentiae* being the most abundant and predominant specie.¹⁵

One study in the USA¹⁶ reported no evidence of contamination of commercial dry dog food with *Dermatophagoides*

during its manufacture or storage. It was suggested that contamination was probably more frequent in warmer geographical regions. Another study also reported no storage mite infestation in dry pet food in Germany, 6 weeks after opening food bags.¹⁷

The aim of this study was to investigate the possible presence of storage mites in different commercial dry dog food, at the opening of the bag and after storing under controlled environmental conditions.

Materials and methods

Sample selection

Ten different commercial dry dog foods (bags of 1.5 kg to 10 kg) presenting different packaging (bag size and closure system) texture, shape and formula of the kibble were selected. All selected foods were premium diets provided by six different manufacturers and formulated for patients with skin condition (four protein-hydrolysed hypoallergenic diets, three limited protein source-based diets, one diet high in fatty acids and two diets aimed to improve skin condition). All bags were placed separately by 30 cm, opened simultaneously and stored open for 6 weeks. In parallel, a duplicate of three of those bags were empirically selected to be opened and stored after being sealed using an adhesive strip (diets 6 and 9) or a zip seal (diet 7) for the same period of time. All samples were manipulated with gloves and sterile material to avoid cross-contamination.

Environmental conditions

Bags were stored in two different locations, and under two different environmental conditions. The duplicate bags were from the same batch.

Condition A

Study performed during the months of March and April 2006, under laboratory conditions. The room where the study was conducted was a clean laboratory with closed windows and not accessible from outdoor.

Condition B

Study was performed during May and June 2006 in kennel conditions. This location was a ventilated garage with outdoor access, situated close to the dog kennels and used to store the kennel material and dog food.

Temperature and humidity in both environmental conditions were recorded daily.

Quantification and identification of mites

At day 0, samples from the 10 bags were collected to analyse for the presence of mites. After different storage periods, samples from the 10 bags stored opened, and the 3 bags stored closed, were collected to perform the following methods for mite quantification and identification.

Food bags stored under condition A were the same bags analysed at day 0, just after being opened.

Microscopic observation

At the time of opening the bags (day 0) and after 3, 7, 14, 21, 28, 35 and 42 days of storage, three samples of 20 g each from the surface, middle and bottom of each bag were taken. The whole food samples were inspected immediately under a stereo binocular microscope (Leica Microsystems GmbH, Wetzlar, Germany). Analysis was performed for 10 min per sample, and examination was always performed by the same investigator.

Guanine determination

Five weeks after opening the bags, guanine concentration was determined. Guanine is the major nitrogenous excretion product of mites, and it has been used as a marker for storage mite allergen in foods.¹⁸ Guanine detection was performed by using a semiquantitative assay ACAREX® Test (Lifestyle Vertrieb, Germany). Briefly, 200 g of the food

Table 1. Results of the initial analysis of food samples by flotation technique

Diet	Number of mites	Types of mites
1	0	–
2	1	Mite fragment (unidentifiable)
3	0	–
4	2	<i>Acarus</i> spp.
5	0	–
6	0	–
7	0	–
8	0	–
9	0	–
10	0	–

samples were taken and vacuumed for 5 min at 800 W. The vacuum filter system to collect dust was thoroughly cleaned between different samples. The dust was weighed and dissolved in phosphate buffer to perform the ACAREX® test, according to the manufacturer's instructions. After 1 min the colour of the test strip was revealed and evaluated following the manufacturer's guide lines (colour scale).

Storage mite traps (BT trap®, Central Science Laboratory, York, UK)

Five weeks after opening the bags, a BT trap, specifically designed for detecting and monitoring storage mite presence, was placed on the top of the food (one trap per food bag), in direct contact with the kibbles and with lid traps unscrewed to allow mite access. Traps were exposed to the food for 6 days. Each trap was then closed and stored in individual polyethylene bags at –20 °C until analysis. This method allows the identification and semiquantification of living mites.^{15,19}

Flotation technique

Food samples (200 g) from the 10 selected food bags were taken at day 0 and 5 weeks after opening the bags. Samples were taken from the surface, the middle and the bottom of each bag and were frozen at –20 °C until analysis. Pet food samples were processed by flotation technique, in order to identify and quantify mites present in each sample. The flotation technique was performed at Central Science Laboratory, York, UK. Samples were thoroughly mixed to obtain a 40-g subsample. One subsample per food was analysed by the method reported by Thind.^{4,20} Collected mites were counted and identified to either family, genera or species level.

Results

Initial analysis of food samples

When bags were opened (day 0) and food samples were immediately analysed by microscopic observation, the presence of mites was not observed in any of the diets. The flotation technique revealed that two of the 10 diets contained mites (Table 1). Sample from diet 2 contained a single fragment of a mite dorsum. The fragment was very badly damaged, with unidentifiable taxonomic features; therefore it was not possible to identify the mite to genus level. Sample from diet 4 contained two storage mites, identified as *Acarus siro*. These mites were observed to be partially turgid, indicating that they may have been alive when the samples were frozen before flotation analysis.

Quantification and identification of mites in stored food samples

This analysis was performed on all bags (10 bags opened and three bags sealed), stored under the two different environmental conditions A and B:

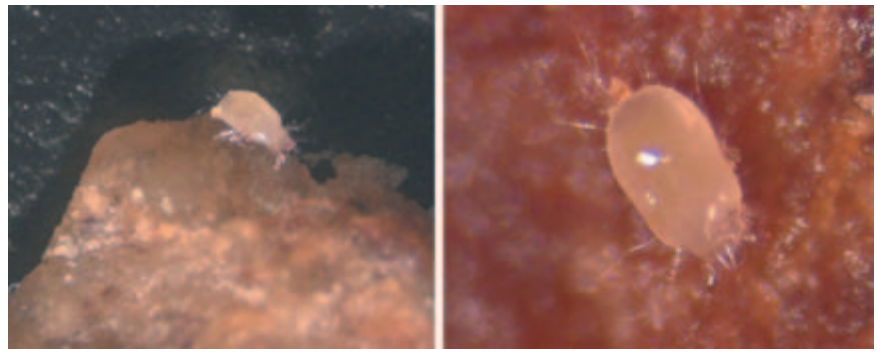


Figure 1. Pet food kibble contaminated by a storage mite.

Table 2. Results of the analysis of guanine test (results expressed as mild, moderate or heavy based on the guanine test colour scale) and storage mite BT traps exposed at day 35 in direct contact with the kibbles, from dry dog diets stored in a kennel (environmental condition B)

Diet	Guanine analysis	Number of mites per trap	Identification
1	Mild	0	–
2	Mild	0	–
3	Mild	0	–
4	Heavy	In excess of 350	Majority <i>Tyrophagus</i> spp. Some mesostigmatic mites
5	Heavy	1	<i>Tyrophagus</i> sp.
6	Heavy	7	4 <i>Tyrophagus</i> spp. 2 mesostigmatic mites 1 tarsonemid
7	Moderate	0	–
8	Mild	1	Mesostigmatic mite
9	Mild	0	–
10	Heavy	27	12 <i>Tyrophagus</i> 11 mesostigmatic mites 4 desiccated storage mites Mesostigmatic mites
6b	Heavy	2	–
7b	Mild	0	–
9b	Mild	0	–

Condition A

Average environmental conditions at laboratory location were 16 ± 2.3 °C and $68 \pm 7.3\%$ of relative humidity.

The presence of mites was not detected by microscopic observation at 3, 7, 14, 21, 28, 35 and 42 days of opening.

Five weeks after starting the study, storage mite traps (BT traps) were placed on the top of all the bags. At the end of the exposure period, the content of each BT trap was inspected. None of the 13 traps were found to contain any mites or insects.

Based on these negative results, the flotation technique and guanine tests were not performed.

Condition B

Average environmental conditions recorded at the kennel were 23.2 ± 2.1 °C and $71 \pm 5.6\%$ of relative humidity.

Microscopic observation

Mite contamination was observed at day 21 in six of the 10 bags stored open (diets: 1, 3, 5, 6, 7 and 10) and in one of the three bags stored sealed with adhesive strip (diet 6b). At day 28, presence of mites was also detected in

Table 3. Results of the final analysis, performed by flotation technique, of food samples from bags stored for 35 days in a kennel garage (environmental condition B)

Diet	Number of mites	Types of mites
1	1	<i>Tyrophagus</i> sp.
2	0	–
3	0	–
4	163	<i>Tyrophagus</i> sp.
5	3	<i>Tyrophagus</i> sp.
6	1	<i>Tyrophagus</i> sp.
7	1	Tarsonemid mite
8	2	<i>Tyrophagus</i> sp.
9	2	<i>Tyrophagus</i> sp.
10	181	180 <i>Tyrophagus</i> spp. and 1 tarsonemid mite
6b	2	<i>Tyrophagus</i> sp.
7b	0	–
9b	1	<i>Tyrophagus</i> sp.

diets 2 and 4. Mite presence was not detected until day 35 in diet 9.

No mites were observed at the end of the study only in three bags: one open bag (diet 8) and two sealed bags (diets 7b and 9b). Image of collected mites is shown in Fig. 1.

Storage mite trap analysis

Results from trap analysis are shown in Table 2. Six from the 13 traps analysed showed presence of mites. Diets 4 and 10 showed the highest number of collected mites (in excess of 350 and 27 mites per trap, respectively), diet 6 showed seven mites and diet 6b two mites, whereas diets 5 and 8 showed the presence of one mite per trap.

Guanine analysis

This assay showed that the highest levels of guanine, a metabolic excretion product of mites, were detected in diets 4 and 10. The rest of the diets showed results from mild to moderate (Table 2).

Flotation technique

After the flotation analysis, 10 of the 13 analysed food bags were found to contain mites (Table 3), the majority of which belonged to the common storage mite genus *Tyrophagus*. Samples from diets 4 and 10 showed the highest quantity of mites per 40 g of food (163 and 181 mites, respectively). All detected mites were partially to

fully turgid, indicating that they may have been alive when the samples were frozen.

Insects or fragments of insects were found in two samples: an insect leg in diet 5 and a single psocid and two insect larvae in diet 10.

Discussion

This is the first report that demonstrates that storage mites can be found in unopened bags of commercial dry food. Dry pet food could be considered a potential source of storage mites due to their nutritional composition.^{6,21} However, the few studies performed to detect mites in dry pet food have shown absence of storage mites¹⁷ or house dust mites.¹⁶ Unfavourable environmental condition for mite productivity was hypothesized to be one of the probable causes of this last study's results.

In the present study, mite contamination was studied in 10 different dry dog food diets stored under different environmental and location conditions for 6 weeks. On opening of the bags, mite contamination was not observed by microscopic observation. However, after flotation analysis, a more accurate analysis, storage mites were isolated from two of 10 diets (diets 2 and 4).

In a recent study, storage mites, mainly *T. putrescentiae*, were captured from the main processing and packing areas of a dry pet food manufacturing factory in UK, even in the cleanest locations.¹⁵ This suggests that pet food ingredients or even the finished products could become a suitable source of storage mites. Although our results obtained after the initial analysis of dry foods did not show a high level of mite contamination, these data indicate that storage mites can be present in packaged dry pet food bags. Moreover, given that detected mites where shown to be partially turgid, this would indicate that they may have been alive when samples were frozen prior to the analysis.

When samples were stored for 6 weeks in a laboratory (condition A), even when environmental conditions (temperature and humidity) became more suitable for mites development, no contamination was observed, probably due to the general hygiene of the location conditions with controlled access to insects and closed windows. A study carried out in the Munich area (Germany) showed similar results, concluding that contamination with storage mites after opening bags of dry commercial dog food was unlikely in this geographical area.¹⁷ Again, the possibility that environmental conditions were not optimal for mite development should be considered.

It is well established that storage mites flourish in a warm and humid environment (optimally at about 25–30 °C; relative humidity of 80%).²² These conditions favour the growth of moulds that provide an important source of food for mites. In the study performed under better environmental conditions for mite growth (condition B), mites were detected by microscopic observation in almost all the dry food bags at 3, 4 or 5 weeks after opening, using the different techniques performed. The chosen location to store bags (kennel garage), probably contained mites, and these multiplied under optimal environmental conditions. Microscopic examination was used as a preliminary method, although this laborious

procedure is considered by some authors to be unreliable, and unsuitable for the early detection of mites.¹⁵ In our study, this method was found to be sensitive and useful to detect mites.

After 5 weeks of storage, diet 4 was found to be the most contaminated samples, as detected by the BT trap analysis and by flotation analysis. In addition, diet 10 was also heavily contaminated. Samples were mostly contaminated by *Tyrophagus* spp.

Besides environmental temperature and humidity, the moisture content and nutritional composition of food have also been considered important factors for mite development. The present results obtained from the two experiments performed under different locations would suggest that environmental factors were more important than moisture content or nutritional composition of the diets.

The study did not enable examination of possible reasons for the differences in the population numbers of mites among dry foods stored under the same kennel conditions. The nutritional content of the two more contaminated diets (4 and 10) was considered to be similar to the other diets in terms of percentage of fat, protein and moisture (data not shown). Packaging was also similar in these two diets. The factor of storing food bags opened could be important. The more contaminated diets were stored opened, which could indicate ready access for mites (and insects). Insects were demonstrated in some of the diets by flotation technique. Conversely, the only food bag presenting an airtight closure and stored sealed with a zip (diet 7b) did not show contamination at any time after storage.

Other relevant data are that the main contaminant mite species found in the different diets was *Tyrophagus* spp., however, mites present in diet 4 once opened were *Acarus* species.

Mites were not detected in diets 2 and 3 by storage mite BT traps, or by flotation technique, whereas mites had been detected by microscopic observation. Probably in these samples mite density was not high and the selected subsamples used in performing the flotation analysis did not contain a detectable number of mites. This result shows that it is crucial to obtain a representative sample from each bag and that the sampling method is also critical to detect the presence of mites.

In the present study, BT traps were used as a first way to detect living mites after microscopic examination. The BT trap has been developed to help in the early detection of pests in different industries. Formulation of the trap lure was specifically designed to attract a wide range of storage mite species.¹⁵ Our study has shown that through this simple method, dry pet food can be easily analysed.

Flotation analysis was also performed to quantify and identify storage mites. This technique has been found to be more sensitive because it detects both living and dead mites, which are a cause of allergy among pets.²³ Flotation technique allows a precise estimation of mite population in infested samples. In our results, the combination of the three techniques (flotation analysis, microscopic observation and storage mite trap analysis) allowed a complete and accurate food inspection. By contrast, results obtained after guanine analysis were not conclusive in our experiment. Guanine detection has been recommended

as a test to quantify the presence of mites; however, insect contamination or other guanine sources different from mites could give false positive results.²⁴ The possibility that the guanine detection method could be more sensitive than the other methods, although unlikely, could also be considered.

In conclusion, this study provides evidence that some commercial dry diets can contain mites even before being opened. It also shows that dry dog food can be a substrate for mite reproduction. It would be necessary to perform more studies to determine if, under defined temperature and humidity conditions, dry pet food bags stored in homes can be contaminated with storage mites. This would give more information to determine whether dry pet food could represent a relevant source of antigens for sensitization in atopic dogs.

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Résumé Les acariens de stockage sont considérés comme des allergènes importants chez les chiens atopiques. Des taux élevés de sensibilisation vis à vis de *Tyrophagus*, *Acarus*, et *Lepidoglyphus* ont été rapportés chez les chiens atopiques et une alimentation sèche a été suggérée comme source potentielle d'exposition à ces acariens. Le but de cette étude était d'évaluer la contamination d'aliments industriels secs pour la présence d'acariens et de déterminer si les conditions de stockage influençaient le risque de contamination. Dix aliments secs premium, formulés pour les chiens à problèmes cutanés, ont été sélectionnés. Les sacs ont été ouverts et conservés 6 semaines dans des conditions environnementales différentes. A différentes périodes, des prélèvements ont été réalisés et analysés par microscopie, test à la guanine, et par une technique de flottaison.

Après ouverture, deux acariens identifiés comme *Acarus siro* ont été isolés d'un des 10 sacs par la technique de flottaison, indiquant que ces acariens peuvent être présents dans les aliments commerciaux. Après 5 semaines de stockage dans des conditions environnementales idéales pour la croissance des acariens (23.2 ± 2.1 °C et 71 ± 5.6 % d'humidité relative), des acariens ont été détectés par observation microscopique dans 9 des 10 aliments. Lorsque les acariens étaient identifiés par la technique de flottaison,

Tyrophagus spp. a été retrouvé le plus fréquemment. Ces résultats suggèrent que les aliments secs peuvent être contaminés par des acariens de stockage et que les conditions de stockage peuvent influencer la contamination des aliments et le développement des acariens.

Resumen Los ácaros de almacén se consideran alérgenos de importancia en perros con dermatitis atópica. Se han observado altos índices de sensibilización a especies de *Tyrophagus*, *Acarus* y *Lepidoglyphus* en perros atópicos, y la comida seca para perros podría ser una fuente potencial de exposición a estos ácaros. El propósito de este estudio fue evaluar comida seca canina comercial para contaminación con ácaros de almacén, y evaluar el efecto del tiempo de almacenamiento y otras condiciones que pudieran influir para el riesgo de contaminación. Se evaluaron diez dietas comerciales de alta calidad formuladas para enfermedades de la piel. Los paquetes de comida se abrieron y almacenaron durante seis semanas en dos condiciones diferentes. Se tomaron muestras a distintos tiempos de cada paquete y se analizaron mediante microscopía, test de guanina y técnica modificada de flotación.

Al abrir las bolsas dos ácaros identificados como *Acarus siro* se aislaron de una de las diez bolsas mediante la técnica de flotación, indicando que los ácaros pueden estar presentes ya en la comida empaquetada. Tras cinco semanas bajo condiciones de almacenamiento óptimas para el crecimiento de los ácaros (23.2 ± 2.1 °C y 71 ± 5.6 % de humedad relativa), se detectaron ácaros al microscopio en 9 de las diez dietas. Mediante la técnica de flotación *Tyrophagus* spp. fue el ácaro contaminante más frecuente. Estos resultados demuestran que la comida seca es un sustrato adecuado para la reproducción de los ácaros de almacén, y que las condiciones ambientales y de almacenamiento pueden influir en el grado de contaminación y en el desarrollo de los ácaros.

Zusammenfassung Vorratsmilben können als wichtige Allergene bei Hunden mit atopischer Dermatitis erachtet werden. Hohe Sensibilisierungsraten auf die Spezies *Tyrophagus*, *Acarus* und *Lepidoglyphus* sind bei atopischen Hunden beschrieben worden, wobei trockenes Hundefutter für eine potentielle Quelle der Exposition zu Vorratsmilben gehalten wird. Das Ziel der gegenwärtigen Studie war es, kommerzielles Hundetrockenfutter auf eine Kontaminierung mit Vorratsmilben zu evaluieren und herauszufinden, wie die Lagerungszeit und -bedingungen die Gefahr einer Kontaminierung beeinflussen können. Es wurden zehn verschiedene hochwertige kommerzielle Hundetrockenfutter, die für Hauterkrankungen formuliert waren, ausgewählt. Die Futtersäcke wurden geöffnet und 6 Wochen lang unter zwei verschiedenen Umgebungsbedingungen gelagert. Zu verschiedenen Zeitpunkten wurden Proben von jedem Sack entnommen und mikroskopisch, sowie mittels Guanin-Test, mit spezifischen Fallen für Vorratsmilben und mit einer modifizierten Flotationstechnik untersucht.

Beim Öffnen der Säcke wurden zwei Vorratsmilben, die als *Acarus siro* identifiziert wurden, aus einem der zehn Säcke mittels Flotationstechnik isoliert, was darauf hinweist, dass Vorratsmilben in verpackten Hundetrockenfuttersäcken vorhanden sein können. Nach 5 Lagerungswochen unter Umgebungsbedingungen, die ideal waren für das Milbenwachstum (23.2 ± 2.1 °C und 71 ± 5.6 % relativer Luftfeuchtigkeit) wurden Milben durch mikroskopische Untersuchung in 9 der 10 Diäten gefunden. Wenn die Milben mittels Flotationstechnik identifiziert wurden, war *Tyrophagus* spp. die häufigste kontaminierende Spezies. Diese Ergebnisse zeigen, dass Hundetrockenfutter ein passendes Substrat für die Reproduktion der Vorratsmilben sein kann und dass die Umgebungs- und Lagerungsbedingungen die Futterverunreinigung und die Milbenentwicklung beeinflussen können.