

Storage mite contamination of commercial dry dog food in south-eastern Australia

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Objective To evaluate contamination of unopened and opened stored sources of commercial dry dog food by viable storage mites.

Design Prospective laboratory and field study.

Material and methods Samples were collected from nine brands of previously unopened bags (new bags) of dry food and 20 field sources of stored dry food in homes in Sydney and Canberra, Australia. All samples were initially examined for the presence of mites using a stereo-binocular microscope and then placed in separate filter-paper-sealed containers. Field samples were incubated at an average temperature of 29°C and 78% relative humidity (RH) for 5 weeks and then at average 26°C/83% RH for 8 weeks. Paired new-bag samples were stored under room conditions (average 23°C/47% RH) and controlled incubator conditions (average 26°C/80% RH) for 6 weeks. All samples were thoroughly examined for mites, mite eggs and visible mould once weekly using a stereo-binocular microscope.

Results Storage mites were not visualised in any of the field samples or in new-bag samples stored at room temperature. Storage mites, identified as *Tyrophagus putrescentiae*, were visualised in increasing numbers in seven of nine new-bag samples after incubation, with first mites and then eggs evident after 3 weeks of incubation.

Conclusions We confirmed the presence of viable storage mites in a range of previously unopened commercial dry dog foods in Australia and confirmed the possibility of heavy storage mite contamination for dry food stored under conditions of moderate temperature and high humidity. These findings have relevance to storage mite and/or dust mite sensitivity in canine atopic dermatitis.

Keywords atopic dermatitis; commercial dry dog food; dogs; storage mites

Abbreviations IDT, intradermal testing; RH, relative humidity

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Storage mites are free-living environmental arthropods that are widely implicated in allergic disease of humans and domestic animals. They belong to the families *Acaridae*, *Glycyphagidae* and *Chortoglyphidae*^{1,2} and are most commonly found in stored cereals, fodder and dry feedstuffs,^{3–5} with protein-rich substrates and mould being their preferred diet.^{2,6} They have been reported to contribute to

atopic dermatitis in humans and dogs, both from unique sensitisation and from cross-reactivity with the more common dust mite allergens.^{7,8} Exposure to storage mite allergens may occur via transepidermal absorption and/or oral ingestion.⁹ Two studies in Spain and Germany demonstrated the presence of *Tyrophagus* species and *Acarus siro* in commercial dry dog foods, with contamination demonstrated in previously unopened bags⁵ or during processing and packaging.¹⁰ Conversely, some studies (Germany¹² and northern USA¹¹) have failed to detect mites in dry dog foods, although the latter study was specifically evaluating house dust mites in dry food rather than storage mites.

Because arthropods are ectothermic, their metabolic activity and development is influenced by environmental temperature, with high rates in warm conditions, and low rates, which facilitate survival, during cold conditions. Accordingly, changes in environmental temperature and humidity are known to affect the growth of storage mite populations.^{6,13,14} Warm and humid conditions enable mite populations to thrive (in general, optimally at 25–30°C and 80% relative humidity (RH)) and also encourage the growth of moulds as a mite food source. Three storage mite species relevant to allergies are *Tyrophagus putrescentiae*, *Acarus siro* and *Lepidoglyphus destructor*, which feed selectively on different mould species and may contaminate their substrates with mould by selectively harbouring spores within their digestive tract or other organs.^{1,15,16} The composition and moisture content of the substrate may also influence mite development.^{5,17} Both the life cycle and relevant environmental conditions have been well studied for *T. putrescentiae*, and *A. siro* (Table 1), with less published literature for *Lepidoglyphus* species and other species implicated in allergies, including *Blomia* species.^{7,8}

To our knowledge, there are no published studies evaluating the presence of storage mites in commercial dry dog foods in Australia. A small pilot study performed by the second author (LJV) revealed the presence of small numbers of storage mites, including identified *T. putrescentiae*, under field conditions in 4 of 49 samples of opened stored dry dog food in Sydney, Australia (unpubl. data).

The objective of this study was to evaluate the presence of storage mites in commercial dry dog foods in Australia when stored under optimal environmental conditions for mite reproduction.

Materials and methods

Field samples

A total of 20 samples of commercial dry dog foods were sourced from 20 homes in Sydney and Canberra, in eastern Australia, between January and March 2010 (Table 2). The sampled dry food was stored

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Table 1. Life cycle and environmental conditions relevant to the storage mite genera *Tyrophagus* and *Acarus*

Storage mite	Life cycle (weeks)	Temperature (°C)			Relative humidity	
		Min. ^a	Optimum	Max. ^b	Range	Optimum
<i>Tyrophagus putrescentiae</i> ^{1,13,14,18,19}	2–3 ^c	10	20–32	35–49	85–100	87–90
<i>Acarus siro</i> ^{1,13,20,21}	2–3 ^c	4	20–25	28–38	62–90	>70

^aMinimum temperature for development; ^bmaximum temperature for survival; ^c6 weeks at lower temperatures.

Table 2. Location and storage details for commercial dry dog food field samples subsequently evaluated for storage mite contamination and local outdoor climatic conditions prior to and during the collection period

Location	Dry food source ^a		Month	Mean temperature (range) (°C) ^b	Mean RH (range) (%) ^b
	Storage site	No. of homes			
Canberra			Oct	6–19 (–2–29)	40–70
	Shed, open bin	2	Nov	12–29 (7–40)	40–80
	Shed, open bag	3	Dec	12–29 (6–39)	30–70
	Laundry, open bag	1	Jan	15–32 (4–40)	40–80
			Feb	15–28 (20–34)	40–80
			Mar	12–24 (9–29)	40–80
Sydney			Oct	14–21 (9–35)	50–70
	Shed, open bin	4	Nov	19–26 (15–40)	50–70
	Shed, open bag	3	Dec	19–25 (14–32)	60–80
	Shed, floor	2	Jan	21–28 (16–41)	50–80
	Laundry, open bag	3	Feb	21–27 (18–38)	60–90
	Kitchen, open bag	2	Mar	19–26 (16–31)	60–80

^aField samples were collected at various times between the months of January and March; one sample was collected from each home.

^bData sourced from Bureau of Meteorology 2010, <http://www.bom.gov.au/climate/data/index.shtml>.

RH, relative humidity

under normal household conditions in open bags or storage containers and included several brands (Hill's[®] Adult Maintenance, Eukanuba[®] Adult Maintenance, Pedigree[®] Adult, Coprice[®] Working Dog, Home-Brand[®] and other unknown supermarket dry foods). Samples included some food spilt on adjacent flooring and some food contaminated by visible mould growth, which was more typically associated with the presence of storage mites in the previous pilot study. Approximately 50 g of food (≈2 heaped tablespoons) from each source was collected into a labelled zip-lock plastic bag; a 10-g portion was initially examined for the presence of storage mites and visible mould spores using a stereo-binocular microscope (see later) and then the sample was under room conditions (not recorded; similar to environmental conditions for new-bag samples detailed later) for up to 2 months until sufficient samples were available to proceed with the incubation stage. Approximately 10 g of each sample was re-examined for mites (see later), then placed in a sterile cylindrical plastic container and sealed using filter paper and adhesive tape, and incubated at average temperature of 29°C and 78% average RH for 5 weeks and subsequently at 26°C and 83% RH for another 8 weeks. Conditions in the incubator were not finely controlled, with a 2–3°C fluctuation in temperature depending on use and room conditions, and some vari-

ation in RH with the use of shallow water trays and sponges to achieve high humidity. Each food sample was inspected weekly on a warmed glass plate under a stereo-binocular microscope. Each entire sample was examined for 10 min by the same observer (CH) and in accordance with the method of Brazis et al.⁵ The numbers of adult mites, eggs, larval and nymph forms visualised in each sample, and the presence of visible mould, were recorded. The glass plate was rinsed, dried thoroughly and warmed between each sample.

New-bag samples

Unopened, new bags of commercial dry dog food were randomly selected, consisting of eight supermarket brands of adult dog food (Pedigree[®] Adult Small Breed, Goodo[®], Coles[®] Complete Balance, Waltham[®] Oral Care, Waltham[®] Weight Management, My Dog[®] Red Small Breed, Purina[®] Lucky Dog Minis, Purina[®] Beneful) and one specialised formula designed for elimination diet trials (Eukanuba[®] Response Fish & Potato). None of the bags had exceeded the recommended expiry date by the end of the study period. All of the selected dog food brands are reportedly produced in Australia from local and imported ingredients. Crude protein content ranged from 22% to 30% and fat content from 9% to 14%.

Table 3. Presence of storage mites, mite eggs, and mould evident on stereo-binocular microscopic examination at weekly intervals of new-bag commercial dry food samples incubated for 6 weeks under controlled conditions of 26°C average temperature and 80% average RH

Dry food sample ^a	No. of mites					Mite identified
	Days 0, 7, 14	Day 21	Day 28	Day 35	Day 42	
1	0	0	0	10 ^b	~100 ^c	<i>Tyrophagus putrescentiae</i>
2	0	3 ^b	6 ^b	11 ^d	~200 ^c	<i>Tyrophagus putrescentiae</i>
3	0	0	6 ^b	9 ^b	31 ^c	<i>Tyrophagus putrescentiae</i>
4	0	1	9 ^b	18 ^c	26 ^c	<i>Tyrophagus putrescentiae</i>
5	0	0	0	3 ^b	4	No
6	0	0	0	0	1	No
7	0	0	0 ^e	0 ^e	0 ^e	—
8	0	0	0	0	3	No
9	0	0	0	0 ^e	0 ^e	—

^a1: Coles Complete Balance (Coles[®]); 2: Purina Lucky Dog Minis (Nestle-Purina PetCare[®]); 3: My Dog Red (Mars, Inc.[®]); 4: Pedigree Adult Small Breed (Mars, Inc.[®]); 5: Goodo (Masterfoods[®]); 6: Waltham Oral Care (Waltham[®]); 7: Waltham Optimum Weight Management (Waltham[®]); 8: Purina Beneful (Nestlé-Purina PetCare[®]); 9: Eukanuba Fish & Potato (Procter & Gamble Pet Care[®]). ^b1–10 eggs; ^c>100 eggs; ^d11–50 eggs. ^eMicroscopically visible mould on dry food.

A clean pair of forceps was used to randomly select two 10-g samples of dry food kernels from each packet immediately after opening the bag. Each sample was inspected for 10 min by the same observer (CB) under a stereo-binocular microscope, looking for storage mites, then placed in a sterile cylindrical plastic container and sealed using filter paper and adhesive tape, as for the field samples. One of each paired sample was stored under room conditions and one was incubated at an average temperature of 26°C and 80% RH. The temperature and RH of the room and for the incubator conditions were recorded using a digital thermometer/hygrometer every 1–2 days and the average values calculated at the end of the incubation period. Samples were stored under room or incubator conditions for 6 weeks each (≈ two mite life cycles). Each entire sample was inspected weekly on a glass plate under a stereo-binocular microscope, as for the field samples, and evaluated for the presence or absence of adult mites, eggs and nymph forms, and for visible mould spores.

Collection of mite specimens

When mites were observed, multiple specimens were readily collected under the stereo-binocular microscope using a fine-tipped new paintbrush and transferred to small sterile vials of 70% ethanol, which were transported to a mite taxonomist for species identification.

Results

Field samples

No mites were observed in the 20 field samples in the first 5 weeks of incubation nor in the subsequent 8 weeks at adjusted incubation conditions. All samples were heavily grossly contaminated with mould and small numbers of psocids (*Liposcelis* spp. or 'booklice': tiny household insects that feed on moulds, fungi and grains) by day 70. Storage and climatic conditions for the two cities where samples were sourced, preceding and during the time of collection, are displayed in Table 2.

New-bag samples

No mites were observed in the 18 dry food samples examined from freshly opened bags on day 0. No mites were observed in the nine dry food samples stored at room conditions during weekly observation from day 0 to day 42.

In the incubated samples, mites were first observed at day 21 (in two samples) and by day 35 in five samples (Table 3). In all samples observed to contain mites, the numbers of mites and eggs increased at each subsequent evaluation. Microscopically visible mould was evident on some samples that did not support mite growth, but was not microscopically or grossly visible on samples that did support mite growth. The gross visual appearance of storage mite-contaminated dry food kernels was unchanged throughout the study period, although heavily contaminated samples gradually changed to a coarse crumble over the subsequent 4–8 weeks, ultimately with loss of all kernel structure.

Average daily laboratory room conditions during this part of the study were 24°C (range 20–25°C) and 47% RH (range 32–60%), and average controlled incubator conditions were 26°C (range 25–27°C) and 80% RH (range 73–87%); however, exact conditions within the zip-lock bags were not recorded.

Adult mites and eggs visualised in all samples had a similar appearance. All adults had white to translucent, glistening bodies with numerous setae. All eggs appeared white, oval and smooth, and were found singly. Collected mites were identified by a mite taxonomist (Dr B. Halliday, CSIRO Entomology, Black Mountain, ACT, Australia) as one species of storage mite, *T. putrescentiae* (Table 3, Figure 1).

Discussion

This study confirms that contamination of Australian commercial dry dog foods with viable storage mites, at least with *T. putrescentiae*, can

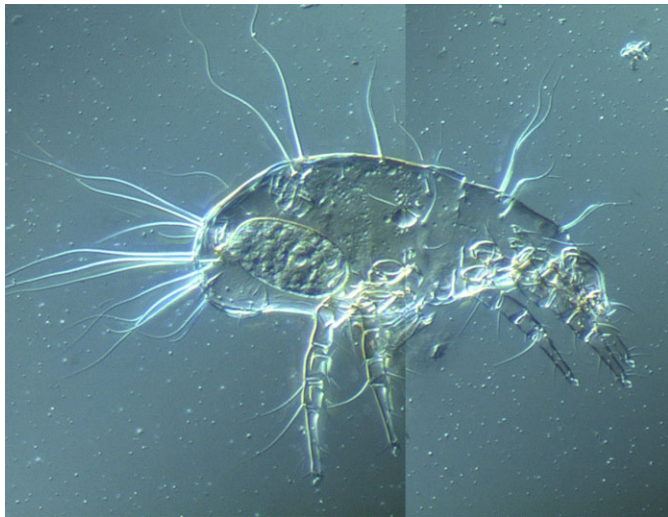


Figure 1. Photomicrograph of a storage mite, identified as *Tyrophagus putrescentiae*, collected from one of the new-bag dry dog food samples after incubation. This specimen is a female, with an egg visible within the abdomen.

occur. Furthermore, rapid mite reproduction producing extensive storage mite populations may occur under suitable climatic conditions and may occur without visible mould or other gross changes to dry food.

Incubator conditions supporting storage mite growth in this study were selected based on optimum conditions reported in the literature.^{5,6,17,18} Environmental humidity and temperature, rather than the nutritional or moisture composition of the dry dog food, are the critical factors influencing mite population growth, development and survival.^{5,13,14,18} Our finding that the incubated new-bag samples exposed to markedly higher RH and slightly higher temperatures led to population growth of mites whereas the paired samples stored under room conditions did not, fully supports this statement.

As mentioned, previous studies of storage and dust mites in dry dog food around the world have provided variable findings and also used different techniques for mite detection. Mites were not detected by a flotation technique using freshly opened dry food samples from bags that had been stored at room conditions for 6 weeks in Germany¹¹ nor using ELISA testing in another study in northern central USA.¹² Conversely, mites were detected using both a flotation method and stereomicroscopic examination of freshly opened and stored samples in Spain, with *A. siro* mites found immediately upon opening the bags, and *A. siro* and *T. putrescentiae* after storage of the food under varying conditions.⁵

Flotation methods are considered more sensitive and specific for detecting storage mites, although are complex, multi-step and time-consuming processes.^{3,5} Stereomicroscopy is widely considered a relatively insensitive method of detecting mites.^{5,10} However, it was considered a practical and rapid method for detecting plentiful mites in the Spanish study,⁵ and its use in the current study facilitated screening of multiple samples more simply than with flotation techniques. It was noted in this study that live mites would move into the

shelter of the food kernels soon after the examination began, immediately after removal from the incubator, which likely contributes to the poor sensitivity of stereomicroscopy. Quick and careful sample handling may facilitate more sensitive results.

Although the mite detection method chosen in this study has limitations, this is most relevant to the absence of mites and thus potentially to the field component of the study as discussed later. Other potential mite detection techniques considered but not available for use in this study included mite traps and ELISA techniques, both of which are rapid tests, with ELISA being the most sensitive and capable of detecting very low levels of mite contamination, including both living and dead mites, mite fragments, and waste products or proteins.¹¹ Guanine (mite waste product) assays are another option, but the potential for false-positive results from dust mites, insects or other guanine sources of contamination is an important disadvantage of this method.^{5,22}

The storage mite contamination of commercial dry dog food detected in this study may have occurred at the raw ingredients stage prior to manufacture and/or during the manufacturing process. It is well established that storage mites may persist in stores of raw grains and cereals.¹ Storage mites, in particular *T. putrescentiae*, have also been detected in the processing and packaging areas of a dry dog food manufacturing plant in the UK.¹⁰ The variable mite contamination of freshly opened dry dog foods around the world may be explained by variations in environmental conditions prior to and/or throughout manufacture, critical to sustaining mite populations in any exposed foods or ingredients. The climate in Sydney and other coastal Australian cities is characterised by moderate average temperatures and periods of high humidity, similar to Spain, which are likely to favour mite survival.

Storage mite contamination of dry dog food may also occur after manufacture, from environmental sources after the bag is opened. However, the apparent low incidence of viable mites in the field samples in Sydney and Canberra in the current study, and in the previous pilot study in Sydney, suggest contamination at or prior to manufacture is the greater risk, at least in these geographical regions.

The absence of mites in the field samples was an unexpected finding and may be related to the poorly sensitive stereomicroscopy detection technique. Furthermore, the apparent preference of mites to hide inside kernels at room temperature could have limited the ability to detect small numbers of mites; examination of field samples after a brief warming may have facilitated mite exposure. The result may also have been influenced by the relatively small sample size and/or the involvement of numerous different collectors and the potential for the small samples collected to be unrepresentative of the entire food bag. Previous studies have shown that obtaining a representative sample from each bag is imperative, especially if the density of the mite population is low.⁵

Measurement of the temperature and humidity conditions for dry food storage had some limitations in this study. Incubator conditions varied through an apparently small range, with relatively crude intermittent manual recording only, and optimal mite growth conditions may not have been consistently provided. This may have affected mite reproduction and population expansion from an initially low level of mite contamination. Additionally, temperature and humidity were not

recorded within the zip-lock bags in which the field samples were stored prior to incubation and it is possible that low RH in particular may have affected mite survival during the storage period.

Another possibility is that the initial high incubation temperature (average 29°C) may have damaged or killed any viable eggs or mites present in the samples. As the microscopic method of detecting mites relies on viable mites or eggs being present, mites that are dead, fragmented or buried within the kernels are less likely to be detected. Although *T. putrescentiae* may thrive at such high temperatures,¹ there are interspecies differences in the optimal temperatures for the mite species previously detected in dog foods,^{1,14} and *A. siro*, for example, may not always survive above 28°C (Table 1). This may have contributed to the fact that *T. putrescentiae* was the only mite species detected in the new-bag samples. The food samples that had identified *T. putrescentiae* contamination did not significantly differ in their protein and fat contents (range 13–24% and 7–10%, respectively (typical analysis)) compared with foods without apparent mites. It seems unlikely that the high protein and fat content of dry dog foods would preclude the development of other storage mite species, such as *A. siro*, which commonly contaminate cheese stores.⁶

It is also possible that, unlike the range of freshly opened dry foods in this study, the field samples were not contaminated with viable mites at or prior to manufacture, or that mites were killed during field storage. The low temperature and/or humidity, both typical of Canberra's climate (Table 2), could have played a vital role in mite survival in this location. Surface populations of storage mites in grain stores are known to be able to 'overwinter' by decreasing their metabolic activity in temperatures as low as 4°C if the RH remains sufficient.^{6,23} However, when the RH is <55%, the rate of water loss from the cuticle in most mite species will exceed the rate of absorption from the environment, and death may result.⁶ Low humidity in Canberra could be the critical factor. In contrast, in Sydney the samples were collected during late summer and early autumn when the temperature and RH would be expected to favour mite growth (Table 2); however, local temperature and RH at the storage sites were not determined and may have varied widely.

However, the absence of mites in the field samples of dry dog food may reflect a true low prevalence of storage mite contamination. Further studies evaluating larger numbers of field samples, with the use of more sensitive mite detection methods if mites remain sparse or undetected, would help to clarify the field prevalence of storage mite contamination of dry dog food stored in homes in eastern Australia.

The ultimate relevance of storage mite exposure for atopic dogs is currently unclear and is complicated by cross-reactivity with dust mites. In one study, 80% of 150 atopic dogs showed at least one positive reaction to one dust or storage mite species on intradermal testing (IDT).²⁴ Only 5% (n = 8) of the 150 dogs reacted to storage mites and not to dust mites, with *T. putrescentiae* the most commonly reactive mite species. In another study, 95% of atopic dogs had serum IgE against *A. siro* and 89% against *T. putrescentiae*.²⁵ In dogs, in vitro cross-reactivity has been demonstrated between storage mite species (*T. putrescentiae* and *A. siro*), and between dust (*D. farinae*) and storage mite species (*T. putrescentiae* and *A. siro*) using ELISA cross-inhibition.²⁶ Immediate-type hypersensitivity has also been demon-

strated to storage mites in vivo in dogs solely sensitised to dust mites.⁹ Thus it appears dogs may experience either parallel or cross-sensitisation to storage mite allergens.^{8,24,26,27} However, the relevance of positive reactivity has been questioned, with some studies detecting similar reactions to *T. putrescentiae* extracts on IDT in both healthy and atopic dogs,⁷ and a high level of storage mite sensitisation is recognised in both healthy and atopic humans.²⁸ Interestingly, dry dog food is also a suitable medium for the growth and reproduction of dust mites (*Dermatophagoides* spp.)²⁹ and exposure on ingestion may contribute to allergic signs in sensitised dogs.¹¹ Several studies have reported that ingestion of dust mite-contaminated foodstuffs directly results in hypersensitivity reactions in humans.^{11,30,31}

Although this study confirmed the potential presence of viable *T. putrescentiae* in dry dog foods, which could rapidly reproduce into large populations, the types and quantities of storage mite antigens required to induce hypersensitivity reactions in atopic dogs, and whether certain processing methods of dog foods can influence their allergenicity,¹⁰ remain uncertain. Regardless, allergen avoidance has been purported as a means of achieving clinical improvement in atopic dogs sensitised to dust and storage mites.^{6,24,32} This is supported by avoidance of dry cat food alone leading to resolution of clinical signs of feline asthma in storage mite-reactive cats in France.³¹ Clinical response to avoidance measures and immunotherapy for storage mites alone in atopic dogs is currently unreported.

Food storage conditions have been found to affect the rate of environmental contamination of human cereal-based foods. Storage in tightly sealed containers, and especially with refrigeration, is associated with low humidity and mite contamination rates, whereas storage in open or poorly sealed containers under room conditions promotes contamination.^{6,11,18,33} It would seem prudent for owners of atopic dogs (and potentially cats) to store any commercial dry food in clean, air-tight containers and refrigerated if possible, particularly in warm and humid geographical regions, and/or avoid humid storage locations (e.g. laundry).

Conclusion

This study has demonstrated that viable storage mites or their eggs can exist in a variety of unopened commercial dry dog foods available for purchase in Australia and that mite populations can rapidly increase with exposure to appropriate environmental conditions of warmth and humidity. These findings may have important implications for atopic dogs with storage mite and/or dust mite hypersensitivity in Australia. Although clinical relevance remains to be confirmed, careful storage of dry dog foods, in sealed containers ensuring low humidity, appears warranted for atopic dogs in eastern Australia with storage and/or dust mite sensitivity.

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