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Revised Proposal for:

Mite Analysis of Dog Food

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Submitted to:

Mark A. Tetrick, DVM, Ph.D.
Research Nutritionist
Research & Development
The Iams Company
Telephone: (937)415-8964

by:

Larry G. Arlian, Ph.D.
Department of Biological Sciences
Wright State University
Dayton, Ohio 45435-0001

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Telephone: (937) 775-2568

Fax: (937) 775-2568

BACKGROUND/RATIONALE

Many species of mites have been found in large numbers in stored grain, hay, and processed foods. Many of these same mite species also thrive on products such as eggs, milk, nuts, beans, animal tissue and yeast that may be ingredients of processed foods consumed by humans and domestic animals. Storage mite allergens and other products from storage mites that are common in stored grains may be carried through the manufacturing process and contaminate the finished product. Likewise, live mites can become packaged in the finished product and multiply during the shelf life of the product. Also, storage mites may find and populate processed foods once the package is opened while in the hands of consumers. Various researchers have reported up to 114 species of storage mites infesting food products (Hughes 1976, Smiley 1983). Pugh (1996) reported 12 species of mites were isolated from 567 samples of cereal-based foods in the UK.

Exposure to house dust and storage mite allergens have caused allergic reactions in humans, including anaphylaxis (Arlian 2002). In the US, an FDA report in 1998 stated that "allergenic mites have been recently identified as an emerging food safety issue by virtue of clinical reports of the discovery that mite allergens may induce anaphylaxis from ingestion of mite-contaminated food" (Olsen 1998). Although four species of allergenic mites (*Dermatophagoides farinae*, *Suidasia* sp. prob. *pontifica*, *Thyreophagus entomophagus* and *Tyrophagus putrescentiae*) have been identified as causing IgE-mediated anaphylaxis when ingested, various related mite species may induce similar systemic reactions when ingested by sensitized individuals (Olsen 1998). Dogs also become sensitized to both house dust and storage mite allergens and atopic dermatitis is common in dogs worldwide (Arlian et al 2003, Schumann et al 2001, DeBorer 1989, Scott 1990, Carlotti & Costargent 1994, Lund et al 1999, Saridomichelakis et al 1999, Hillier & Griffin 2001). Dogs with atopic dermatitis commonly give positive intradermal skin tests to dust and storage mites and have serum IgE against many allergens of these mites (Wellington et al 1991a,b; Sture et al 1995; Saridomichelakis et al 1999; Masuda et al 1999, 2000; Arlian et al 2003). A recent study of sera from 84 dogs with atopic dermatitis residing in various regions of the United States and Europe found that 94% had serum IgE to one or more of the storage mite species that were tested (*Tyrophagus putrescentiae*, *Acarus siro* and *Blomia tropicalis* (Arlian et al 2003). In the Arlian study, 95, 92 and 89% of the storage mite-sensitive dogs had serum IgE against proteins in extracts of *A. siro*, *B. tropicalis* and *T. putrescentiae*,

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respectively. Eighty-two percent had serum IgE against at least 1 protein in all 3 species.

While the source of mite exposure in dogs is not well documented, similar to humans, dogs living in agricultural settings may be exposed to stored product mites and house dogs may be exposed to house dust mites in their homes. House dust mites and storage mites have been reported to occur in dog and cat beds (Eaton et al 1985). In addition mites and their products may contaminate dry dog food and ingested or inhaled storage mites may sensitize and/or cause allergic reactions in dogs as they do for humans. Some commercial dog foods serve as excellent growth media for mites (Arlian et al unpublished). Therefore, it is possible that mites may infest and contaminate dog food used for pet consumption and could sensitize or cause allergic reactions in the dogs that eat the contaminated food.

Mite allergens could contaminate pet food from two main sources. Mites and/or mite allergens could be present in the grains, meat meals or other ingredients used to manufacture the dog food or they may be introduced during manufacture. Alternatively mites could grow in the bag after processing during storage or they may infest the diet after the bag is opened in the home of the consumer.

A significant proportion of dogs considered to have atopy could be responding to mite allergens in their diet. Atopy in pets is largely diagnosed by exclusion. One of the exclusions is food allergy that is deduced when dogs improve when placed on a novel protein diet. The presence of mite allergens in exclusion diets could confound these trial diets. A dog may not improve on a novel protein diet not because the dog is allergic to the novel protein but because it contains mite allergens. Many food allergic dogs could be misdiagnosed because they don't improve on exclusion diets containing mite allergens. The improved success of home made diets in canine food allergy trials may be due in part to the lower levels or absence of mites in the human grade ingredients used.

Surveying commercial pet food for mites and mite allergens at the time the bag is opened and also determining if mites infest and grow in significant numbers once the bag is opened are first steps to determining if dog food is a source of mites/mite allergens that sensitize dogs. If mites/mite allergens are detected in the bag at the time it is opened, the source of the mites and/or allergens can be traced back through product storage to the point of extrusion and back to individual ingredients if needed to determine where mite allergens are entering the product.

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Current methods for detecting storage mites in processed foods are limited and not well developed. They include sieving (Wilkin 1982), flotation analysis (Thind 2000), measurement of guanine content (Bischoff et al 1989) and use of baited traps (Thind 1997). Because of the limitations with the current methods monitoring for mites in stored commodities is not undertaken with thoroughness (Dunn et al 2003). Immunoassays are an approach because they are based on the detection of specific antigens by antibodies, they are sensitive and they are reliable and rapid (Dunn et al 2003). Immunoassays have been developed for a number of applications including detecting the grain weevil in wheat (Chen and Kitto (1993). Brader et al (2002) developed an immunoassay for a variety of common insect pests of both grain and flour. There are also immunoassays for potato cyst nematodes (Dunn et al 1995), field kits for several plant viruses (Danks and Barker 2000) and an ELISA method for house dust mites (Lucynska et al 1989). There have been few studies on developing immunoassay methods for storage mites. Preliminary ELISA studies using *Lepidoglyphus destructor* and *Acarus siro* have been reported (Härfast et al 1996, Dunn et al 2003). The studies look promising but they lacked sensitivity in the presence of grain. The FDA in 1998 in establishing regulatory action criteria stated that "future research needs include development of methods for direct testing of food products for mite allergens."

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OBJECTIVES

The purpose of this study is to determine the presence/absence of storage mites in a variety of commercially available dog foods. The testing will provide answers to the following questions:

1. Do commercial dog foods have significant mite and/or mite allergen contents to potentially sensitize dogs and/or induce allergic reactions, and/or
2. Do the mites and allergens develop in the products while in the hands of consumers?

METHODS

For this study, ~30 different dog foods will be purchased and evaluated. Berlese extraction and immune assays will be used to determine the presence/absence of storage mites and their allergens. In addition, ~50-75 opened bags will be purchased from consumers representing a cross section of storage conditions. These will be assayed also for storage mites. Also, bags of dog food free of mites will be placed in homes, then mite levels will be determined when the bag is almost empty.

Initial experiments will include spiking new bags of dog food with storage mites to evaluate the sampling methods and quantitation of mites and mite allergens.

Dog Foods

Various types/brands of dog food (mutually agreed upon by Iams and Dr. Arlian) will be purchased and will include the following:

1. Unopened Dog Food Bags

Approximately 30 types/brands of dog food (new and unopened) will be purchased from a grocery or pet store for analysis.

Different lots of identical bags of dog foods that were manufactured at different times of the year (representing different climatic conditions during manufacture) will be purchased and the analyses will be repeated quarterly over the period of a year.

2. Random Sample of Dog Food from Consumers

Approximately 50-75 bags of dog food (opened several months previously and used to feed their pets) will be purchased from consumers. Dog owners will be solicited by e-mail at Wright State University during the summer. Each participant will be asked to provide their stored bag of dog food and asked to

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complete a questionnaire specifying the brand of dog food, approximately when it was purchased and opened and the storage conditions (inside, outside or garage; unsealed, loosely sealed or tightly sealed) (DeBoer and Schreiner 2001). This sample will provide us with a random representation of the dog food that consumers are feeding their pets that are stored in a variety of conditions during the warm, humid summer months when mites are most likely to grow in them.

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3. Monitored Conditions Utilizing Dog Food from Consumers
Selected participants (from No. 2 above) will be provided with a new bag of dog food (duplicate bag from No. 1 above) during mid summer (high mite season). It is expected that only a few brands/types will be included in most (~80%) of the dog food provided by the participants (No. 2 above) in the summer. Twenty participants utilizing one of the five most common brands/types will be provided with a bag of their usual brand of dog food. They will be asked to feed from that bag and store it in their usual manner. The bag will be retrieved in late summer for analysis. This sample will provide us with corresponding information for brands/types and lots of food in which the mite and allergen content is known before it is placed with the consumer (in other words confirmed that it was mite free before it went into the home).

Testing of newly opened and previously opened (by consumers) bags of dog food

1. Berlese extractions for collection of live mites (White et al 2002).
Half (the remaining half will be extracted for immunoassay) of each bag dog food will be placed into a Berlese funnel under a 25-watt lamp and the heat will cause any live mites present in the food to crawl out and drop into a container (containing 70% ethanol) below. The total number of mites collected will be determined. In addition, a sample of live mites will be removed, mounted and identified to species using a stereomicroscope.
2. Development of an immunoassay to detect storage mites in dog food.
Tyrophagus putrescentiae (TP) (the most common stored product mite) and either *Acarus siro* (AS) or *Lepidoglyphus destructor* (LD) antisera will be produced in rabbits by our standard methods in place in our Laboratory Animal Resources facility. The TP and LD or AS antisera will be used for either SDS-PAGE/immunoblot analysis or ELISA, whichever technique we are able to develop to detect stored product mite antigens in dog foods. For both methods we will use the rabbit antisera in an immuno-affinity batch extraction to concentrate and elute the storage mite antigens that are present in extracts made from the dog foods to be tested. The antigens that are eluted from the column can then be detected on an SDS-PAGE/immunoblot. A second possible detection method will include an ELISA using the rabbit antisera for detection of storage mite antigens (may need affinity purification) in extracts made from dog foods.

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Timeline

Following approximately 6 weeks to produce the rabbit antisera (it may be longer if boosting is needed), the mite immune analysis can begin. While the antisera are being produced, we will determine the quantitation with the Berlese extraction method. The budget also includes some time we spent determining what species of storage mites could live on dog foods (this has been about 5 months at 80% time but only 1 month of this is in this budget). Therefore, the budget is for a 14-month study.

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