

Aeroallergens in Dairy Barns near Cooperstown, New York and Rochester, Minnesota¹⁻³

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Introduction

Farmers who work in barns frequently develop occupational asthma or hypersensitivity, and other allergic patients often have acute symptoms when they visit barns (1-7). There are many sources of allergens in barns: the animals and their feed, weeds, molds and actinomycetes, rats and mice, insects, and mites. British and Scandinavian investigators have addressed the issue of allergy in the barn in a number of recent studies (3-5, 8). With rare exceptions (9), their conclusions regarding aeroallergens have been based upon indirect evidence derived from skin testing, radioallergosorbent test (RAST), and antigen challenges rather than direct measurements of barn air. The prevalence of a number of potential allergens in the barn atmosphere has not been studied with immunochemical methods previously. Therefore we sampled barn air in 16 barns and measured various aeroallergen levels.

Methods

Air Sampling

Ten barns in Cooperstown, NY and six barns in Rochester, MN were selected for sampling by methods previously described (10). They were representative of large commercial dairy barns, containing an average of 30 to 50 cows, each in an individual stall. The stalls were arranged in two rows running the length of the barn. The rows were separated by a six- to eight-foot central walkway with gutters for manure removal on either side. The animals faced outward, away from the central area, and the feeding of the animals took place along the two lateral walkways—typically about four feet in width. All the barns had cement floors and hay or straw was used as bedding in the animal stalls. A variable number of windows lined the side walkways and ventilating fans were located either in the side walls or at the door at both ends of the barn. Most of the sampled barns had 7 to 8 foot ceilings with large amounts of hay stored in bales on the floor directly above the animals. Two or more upright silos were attached to each of the barns by a small "silo shed" or "feed room." Inside of each barn in Coopers-

town, two volumetric air samplers (Air Sentinel™; Quan-Tec-Air, Inc., Rochester, MN) were placed at opposite ends on the main floor. The Rochester barns were sampled using a single air sampler placed in the middle of the barn. Samples were collected for 20 or 60 min at an average flow rate of 3 L/s. A second sample was collected at the same location several months later. Although feeding of moist silage had recently occurred in several barns, no overtly dusty operations were underway at the time of the sampling in any of the barns.

Particles in the air were collected on a polytetrafluoroethylene (PTFE) membrane with a pore size of 0.3 μ m (Quan-Tec-Air, Inc.). Filter membranes were stored in individual sealed bags. The bags were opened and the filters placed on the air sampler only seconds before sampling began. Upon termination of sampling, the filters were immediately removed from the sampler, placed back in the bag, and promptly sealed. All bags and filters were numerically coded for later identification of specific barns. After collecting the sample, the fibrous backing on the PTFE membrane was stripped away and the membrane extracted overnight in 2 to 3 ml of 0.1 M phosphate buffer, 0.2% bovine serum albumin, pH 7.6 and 50% glycerine. The sample was then decanted into a Brinkmann vial (Brinkmann Instruments, Westbury, NY) and centrifuged at 10,000 rpm for 5 min to remove the solid particles. The supernatant was then decanted into a clean vial and stored at -20° C until analyzed.

Allergens

The following were obtained as National In-

SUMMARY We sampled atmospheric barn air using a volumetric air sampler in ten barns near Cooperstown, NY and six barns near Rochester, MN, and, with radioimmunoassays, measured allergens of *Aspergillus fumigatus*, *Thermoactinomyces vulgaris*, *Micropolysporium faeni*, short ragweed rye grass group I pollen, *Alternaria* (Alt-1), *Dermatophagoides* sp., *Lepidoglyphus destructor*, common insect allergen, mouse urine, rat urine, and cattle epithelium. The most abundant allergen present was *A. fumigatus* followed by *L. destructor*. This study provides initial data on barn aerobiology and demonstrates for the first time the abundance of *L. destructor* allergens in North American dairy barns. More comprehensive study of barns, poultry houses, confinement houses for swine and other agricultural environments from various geographic locations is needed to define the allergen levels to which millions of farm workers are exposed each day. While most of the allergens were expected, the presence of airborne allergens reactive with antisera to *Dermatophagoides* suggests indirectly that substantial amounts of pyroglyphid mites are present in some barns.

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stitutes of Health Research Reference agents: *Aspergillus fumigatus*, *Micropolysporium faeni*, and *Thermoactinomyces vulgaris*. The *Alternaria* (Alt 1) preparation was from Dr. John Yunginger (11). The rye grass pollen was obtained by manual excision of mouse bladders. The urine was lyophilized against 50 mM ammonium bicarbonate using 3,500 MW cutoff Spectropore (Spectrum Medical Industries, Inc., Los Angeles, CA). The retentate was then lyophilized. Rat urine was collected in metabolic cage separated the urine from the feces and processed in the same manner as the mouse urine. Rye grass pollen was obtained from Hollister-Stier (Spokane, WA). The pollen was defatted for 4 h with ether in a Soxhlet extractor (Curtin Matheson Scientific, Eden Prairie, MN) and dried at room temperature. The pollen was extracted (1:10, w/v) in 200 mM ammonium bicarbonate over 24 h at 4° C. The pollen extract was dialyzed against 50 mM ammonium bicarbonate.

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3,500 MW cutoff casing. The dialyzed extract was then lyophilized, assayed for rye Group I allergen content and was assigned 1.7 mg/ml. Short ragweed (SRW) allergen preparation was obtained from Greer Laboratories (Lenoir, NC). It was calibrated in international reference standard units (IU), and contained 217 antigen E units/ml. *Dermatophagoides pteronyssinus* preparation was obtained from Hollister-Stier. It contained 117 µg antigen Der pI equivalents per ml as per Dr. Martin Chapman, University of Virginia (12) or 7.2 IU/ml. The cattle epithelium extract obtained from Hollister-Stier was labeled 1:50 (wt/vol) and was 50% glycerinated.

Frozen, whole-body German cockroaches (WBGC), *Blattella germanica*, were obtained from Hollister-Stier. The WBGC were flash frozen in liquid nitrogen, lyophilized, detatted for 4 h with ether in a Soxhlet extractor, crushed in a mortar and pestle, and extracted 1:10 (wt/vol) in 200 mM ammonium bicarbonate overnight at 4° C. The cockroach extract was clarified by centrifugation and dialyzed against 50 mM ammonium bicarbonate using 3,500 MW cutoff casing. The dialyzed extract was again clarified by centrifugation and divided into two equal aliquots. To one half, an equal volume of glycerine was added while the other half was lyophilized. Protein content of the glycerinated extract was 10 mg/ml by the method of Lowry (13). The extracts were stored at 25° C. Common insect allergens (CIA) were obtained by applying the lyophilized WBGC extract over a Sephadex G-200 column (Pharmacia Fine Chemicals, Piscataway, NJ). Three pooled fractions were obtained from the column. The second fraction (protein content 12 mg/ml) containing molecular weights greater than 55K was broadly cross-reactive by radioimmunoassay with insects from the orders *Lepidoptera*, *Diptera*, *Hymenoptera*, and *Ephemeroptera* (14, 15). Hence, this fraction is designated CIA.

Storage mites (*Lepidoglyphus destructor*) were obtained from Allergon Inc. (Uppsala, Sweden). Whole-body mite was defatted for 4 h with ether in a Soxhlet extractor, and dried at room temperature. Defatted storage mite was crushed with a mortar and pestle, and extracted (1:10, wt/vol) in 200 mM ammonium bicarbonate for 48 h at 4° C. The storage mite extract was clarified by centrifugation, and dialyzed against 50 mM ammonium bicarbonate using 3,500 MW cutoff casing. The dialyzed extract was again clarified by centrifugation, and divided into two equal aliquots. To one-half, an equal volume of glycerine was added while the other half was frozen. Protein content of the glycerinated extract was measured at 1.3 mg/ml by bicinchoninic acid method (Pierce Chemical Company, Rockford, IL).

Antibodies

Human antibody pools containing IgG were used in the assays to quantitate *A. fumigatus*, *M. faeni*, *T. vulgaris*, and *L. destructor*. Serum from two to eight individuals with

farmers' lung or allergic aspergillosis were used. The antibody to *L. destructor* was found by screening 48 sera from farmers' lung patients with *L. destructor*, *Acries siro*, and *Tyrophagus putrescentiae*. IgE antibody pools from two to eight individuals who were sensitive to a specific allergen and who had not received immunotherapy were used in the assays to quantitate Alt 1, mouse urine, rat urine, rye grass pollen, and SRW. The assay for CIA used sera containing IgE from two patients. While both patients had summer and fall rhinitis and positive RAST to moth (*Pseudaletria unipuncta*), one had rhinitis associated with swarms of lakefly (*Chironomus plumosus*) plus a positive RAST and skin test to midge (serum provided by Dr. Steven Kagen). Neither was clinically sensitive to insect venoms, or had been on immunotherapy of any kind, or had frequent exposure to insects in his occupations or hobbies. The IgE antibody used for the cattle epithelium allergen was obtained from a veterinarian who had occupational asthma from cows.

The mite assay employed a polyclonal rabbit anti-*D. pteronyssinus* serum obtained from ALK Laboratories (Copenhagen, Denmark). Rabbit anti-mite antibodies were adsorbed to microtiter plates as the capture antibody. The detection antibody used a radioiodinated affinity-purified preparation of the same serum. Affinity-purified antibody was obtained by passing the rabbit serum over a column measuring 75 cm in length by 15 mm in diameter containing 1 g activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) to which *D. pteronyssinus* (ALK Laboratories) allergens were coupled. After washing the column free of unbound protein, the antibody was eluted with glycine buffer pH 2.3. The fractions from the column were measured for spectrophotometric absorption at 277 nm and a pool was made with the highest absorbing fractions.

Radioimmunoassay

Most samples were assayed by inhibition analysis using a microtiter plate procedure. Such was similar for all allergens, except that the sensitivity varied for each system. The microtiter plate assay procedure was as follows: 100 µl of allergen containing 1 µg of protein was added to an Immulon-2 (Dynatech Laboratories, Alexandria, VA) well, which was then incubated overnight in a humidity chamber at room temperature. The plate was washed three times with RAST wash buffer using a Nunc Immuno-wash 12 plate washer (Nunc-Inter Med, Copenhagen, Denmark). A standard inhibition curve was generated with a reference allergen standard. To each well, 50 µl of test allergen (standard or unknown) was added followed by 50 µl of serum containing appropriate antibody. The plate was gently agitated for 1 min and allowed to incubate overnight at room temperature in a humid box. It was washed and the wells were filled with 100 µl containing 100 ng of radioiodinated ¹²⁵I human anti-IgE, or for the IgG antibody systems, radioiodinated *Staphylococcus*

Protein A (Genzyme Corporation, Boston, MA). After a second overnight incubation, the plate was washed and each well counted in a gamma counter.

The two-site pyroglipid mite assay was performed in the following manner: 100 µl of a 1:500 dilution of the polyclonal rabbit anti-*D. pteronyssinus* rabbit antibody was adsorbed to each well at pH 9.2, 20 mM carbonate buffer. This solution was incubated overnight at room temperature. Again the plate was washed, and to each well 50 µl of test allergen (standard or unknown) was added followed by 50 µl RAST diluent. The plate was gently agitated for 1 min then allowed to incubate overnight in a humidity chamber, at room temperature. It was washed and the wells were filled with 100 µl (20 ng) of radiolabeled ¹²⁵I affinity-purified rabbit anti-*D. pteronyssinus* and the wells from the microtiter plate were counted in a gamma counter. Although this assay was expressed in terms of antigen Der pI equivalents from a reference standard of *D. pteronyssinus*, extracts of *Dermatophagoides farinae* cross-react extensively. The assay measured allergens from *Dermatophagoides* and related genera. Storage mites, *Lepidoglyphus*, *Acarus*, and *Tyrophagus* did not cross-react in this assay.

Microscopic Identification and Quantification of Mites in Barn Dust

After collecting the dust from representative barns, the sample was transported to the laboratory in a sealed Petri dish and stored in the refrigerator at 5° C. Dust samples were sieved using a round copper sieve (mesh size 20) and a copper reservoir of the same size. The fine sample recovered from the reservoir was set aside for further studies. One hundred milligrams of the sample was placed in a 100 ml beaker to which was added 25 ml of lactic acid and held overnight at 45° C. After incubation, the beaker was then placed on a maxi-mix for 2 min and rotated to form a suspension of mites and dust. After 5 min (enough time to allow larger particles to settle), five 5-ml aliquots of supernatant suspension containing the mites were placed into small Petri dishes (60 × 15 mm; Falcon, Oxnard, CA) and the mites were examined under a dissecting stereoscope at × 25. All the mites present in each aliquot were mounted on microscope slides in Hoyer's medium for identification and counting. The number of mites in each sample was determined and recorded as mites/g of reservoir dust.

Results

The levels of aeroallergen were quite similar in barns near Cooperstown, NY and Rochester, MN (figure 1). For comparison we have provided information about the previously measured maximal levels of some of these allergens in other typical locations (table 1). The concentrations in barn air were quite substantial, often many times higher than other locations.

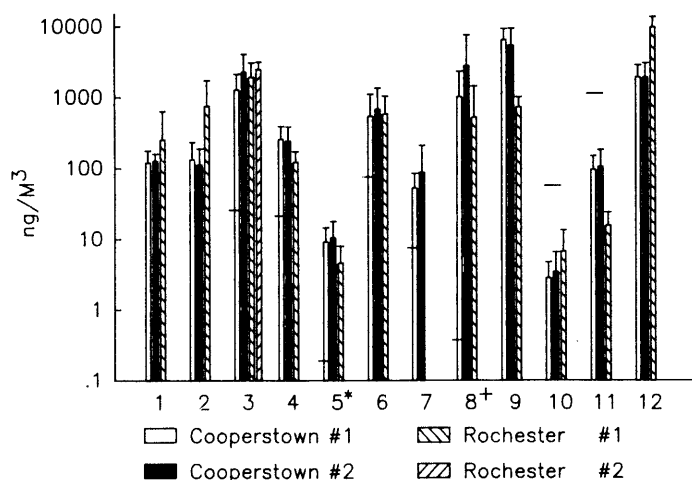


Fig. 1. Average aeroallergen levels and standard deviations from dairy barns in Cooperstown, NY, Sample 1(□), Sample 2(■), and Rochester, MN, Sample 1(▨), Sample 2(▩). All units are ng/m³ except number 5 and number 8. Single horizontal lines denote maximal allergen levels obtained in other typical locations (see table 1). (1) *Micropolyspora faeni*; (2) *Thermoactinomyces vulgaris*; (3) *Aspergillus fumigatus*; (4) *Alternaria alternata*; (5) *Ambrosia artemisiifolia*, (IU/m³); (6) *Lolium perenne*; (7) common insect allergens; (8) *Dermatophagoides pteronyssinus*; (μg Der p1 antigen equivalents × 10⁻⁶/m³); (9) *Lepidoglyphus destructor*; (10) *Mus musculus*; (11) *Rattus norvegicus*; (12) *Bos domesticus*.

Simultaneous samples taken at each end of the Cooperstown barns were nearly identical and samples from Rochester taken several months apart were also very similar.

Dust from five of the ten barns (Cooperstown) was collected and examined microscopically for mites. In Barn 1, the predominant species was the acarid mite, *Caloglyphus berlesei*. Other mites found in smaller numbers were *Euroglyphus maynei*, *Lepidoglyphus destructor*, and *Blomia* spp. In Barn 2, the predominant species was *Chortoglyphus arcua-*

tus. Other species present were predator mites of the genus *Cheyletus*. In Barn 3, the predominant mite species was *Glyciphagus (Myacarus) ornatus*. Other mites present in smaller numbers belonged to the family *Pyemotidae*, which are known to produce severe urticarias in affected farmers. The hypopi (immature forms of *Glyciphagus ornatus*), were also present and belonged to the pilicolous type and are normally attached to the hairs of rats. In Barn 4, the predominant mite species was *Caloglyphus berlesei*, present in adult and hypopi stages. In Barn 5, the

predominant mite species were *Acisiro* and *Tyrophagus putrescentiae*, present in all stages. Pyroglyphid mites were present in only one sample (*Euroglyphus maynei*, Barn 1). Mites were commonly found in very large numbers. In all cases, large colonies were present more than 10,000 mites/g of dust could be counted.

Discussion

It was not surprising to measure concentrations of aeroallergens in dairy barns; perhaps some of the other results were less predictable. Proteins reactive with antibody to thermophilic actinomycetes were measured in undisturbed barn air. This was our experience in measuring these antigens, and we have no basis for interpreting the significance of this concentration in undisturbed barns. Episodes of farm lung usually follow activities that are extremely dusty, such as opening silos or spreading moldy bedding.

Detection of antigens cross-reacting with *Dermatophagoides* was unexpected, for dairy barns are not infested with mites of the genus *Dermatophagoides* (16-19). Presumably antigens from related genera cross-react with *Dermatophagoides* although the only pyroglyphid species found in the 5 barns we examined was *Euroglyphus*. The importance of mites other than *Dermatophagoides* in causing allergic disease deserves further study.

Patients with farmers' lung develop antibodies to many airborne antigens, including thermophilic actinomycetes, so it is not surprising to find a patient with antibodies to *L. destructor*. We found a patient with antibodies to *A. siro* and *putrescentiae* suggesting that they are abundant in the dairy environment. We did find airborne antigens reacting with antibodies to *L. destructor* in what seemed to be high concentrations. This is a significant though not surprising finding since *L. destructor* and possibly other storage mites are increasingly recognized as an important cause of allergic symptoms in farm workers (3, 4). Our search for IgG or IgE antibodies to other pyrophagous mites, *A. siro* and *T. putrescentiae*, in sera of patients with farmers' or barn dust asthma has not, as provided a reagent for assay of the allergens. Although Von Hage-Han (20) described some cross-reactivity between *T. putrescentiae* and *L. destructor* and in some systems cross-reactivity properties have been found between *matophagoides* and *Tyrophagus*, ex-

TABLE 1
MAXIMAL CONCENTRATIONS OF AEROALLERGENS MEASURED IN REPRESENTATIVE LOCATIONS

Agent	Concentration	Location
1. <i>Micropolyspora faeni</i> *	2.727 mg/m ³	Cooperstown barn while operating a bedding chopper
2. <i>Thermoactinomyces vulgaris</i> *	187 μg/m ³	Cooperstown barn while operating a bedding chopper
3. <i>Aspergillus fumigatus</i>	26 ng/m ³	House with decaying studs and moldy insulation in walls
4. <i>Alternaria alternata</i>	25 ng Eq/m ³	New York tenement
5. <i>Ambrosia artemisiifolia</i>	0.20 IU/m ³	Rochester outdoors in August
6. <i>Lolium perenne</i>	85 ng/m ³	Rochester outdoors in June
7. Common Insect Allergen	8 ng/m ³	Rochester outdoors in July
8. <i>Dermatophagoides pteronyssinus</i>	0.4 ng Ag Der p1 Eq/m ³	Rochester house
9. <i>Lepidoglyphus destructor</i> *	12 μg/m ³	Cooperstown barn
10. <i>Mus Musculus</i>	60 ng/m ³	Mayo Animal Quarters
11. <i>Rattus norvegicus</i>	1200 ng/m ³	Mayo Animal Quarters
12. <i>Bos domesticus</i> *	16 μg/m ³	Rochester barn in winter

* Not determined in any environment other than the barns.

from these mites do not cross-react with the human or rabbit antibodies to *Dermatophagoides* or *Lepidoglyphus* that we used.

The concentration of *A. fumigatus* was extremely high. The highest value we have measured to date in other environments is 25 ng/m³ in an obviously moldy house. Levels in most houses are less than 5 ng/m³. Thus, barns appear to be an important source of exposure to *Aspergillus*. *Alternaria* concentration in barn air were also an order of magnitude higher than maximal outdoor concentrations as were the rye grass and ragweed pollen allergens. On the other hand, mouse and rat urine proteins, although present, occurred at concentrations much lower than in laboratory animal care rooms and appear unlikely as an important cause of allergic diseases in dairy farmers.

The common insect allergen assay measures allergenic epitopes widely distributed among insect orders. Concentrations of these insect allergens vary considerably between barns but occasional barns were tenfold higher than outdoor air or air in Rochester houses and were in the range of concentrations found in tenement dwellings of Harlem, a neighborhood of New York City where cockroach-induced asthma is a common problem.

This study demonstrates the feasibility of directly measuring aeroallergen levels in agricultural work environments using radioimmunoassays. These measurements reflect the first attempt to quantify directly the levels of a number of common allergens in the barn environment.

A. fumigatus and mites appear to be the most prevalent and abundant aeroallergens followed closely by cattle epithelium, *M. faeni*, and *T. vulgaris*. Next most abundant allergens were the rye grass group I pollen, *Alternaria*, and rag-

weed pollen. These concentrations are higher than maximal concentrations outdoors so they are likely to provoke allergic disease in subjects allergic to these allergens who enter barns. The lowest allergen levels were the rat and mouse urines and the common insect allergen, and they seem unlikely to cause disease in dairy workers.

Although a total of 16 barns in two geographically diverse areas were studied, we still have a very incomplete understanding of dairy barn aerobiology. Detailed studies of dairy barns in other climates coupled with personal air samplers of farm workers while they are performing specific tasks need to be correlated with symptoms and individual IgE antibody assays.

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