

The prevalence of *Dermatophagoides* mite allergen in Colorado homes utilizing central evaporative coolers

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Objective: The prevalence of mite allergen was studied in homes in which the indoor relative humidity may be increased by use of an evaporative cooler.

Methods: Colorado homes in which central evaporative coolers are used and control homes were evaluated. ELISAs with monoclonal antibodies specific for Der p 1 and Der f 1 were performed on dust samples from each home in May and August. Indoor relative humidity and temperatures were recorded daily.

Results: May samples did not show significant levels of mite allergen ($<2 \mu\text{g/gm}$ dust). Of the August samples, 48 of 95 samples from homes with evaporative coolers (50.5%) had levels of Der p 1 and Der f 1 of $2 \mu\text{g/gm}$ dust or greater, but only 5 of 95 control samples (5.2%) had levels of $2 \mu\text{g/gm}$ dust or greater ($p < 0.00001$). Twelve of 19 homes with evaporative coolers (63%) were positive for mite allergen. Five of 19 (26%) control homes were positive ($p < 0.05$). The homes with evaporative coolers had average indoor relative humidity of 51% or greater; control homes had average relative humidity of less than 45%.
Conclusion: Altering the indoor environment by raising indoor relative humidity through use of evaporative coolers leads to conditions that may facilitate *Dermatophagoides* survival. (*J ALLERGY CLIN IMMUNOL* 1995;96:473-9.)

Key words: *Dermatophagoides*, dust mite, Colorado, evaporative coolers, humidity

House dust mite allergens are well established exacerbants of asthma, allergic rhinitis, and atopic dermatitis. Dust mites are considered to be important perennial allergens for patients who demonstrate sensitivity to mite allergen and have been implicated in the pathogenesis of asthma.¹⁻⁹ Dust mite survival is dependent on appropriate environmental conditions. Temperature and relative humidity are the major factors that influence mite proliferation and survival.^{10, 11} The critical equilibrium humidity for the dust mite is the relative humidity below which the dust mite gradually

Abbreviation used

BBS: Borate-buffered solution

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dehydrates and dies. Survival time of dust mites under dehydrating conditions is primarily influenced by the temperature and relative humidity. Critical equilibrium humidities for *Dermatophagoides pteronyssinus* and *D. farinae* have been determined to be 73% and 70%, respectively (at 25° C).¹⁰ Mite populations fluctuate with seasonal variations of relative humidity. Peak levels occur during the humid summer months (July through October), whereas profound decreases in mite counts occur during the dry season (December through April).¹⁰⁻¹⁶

Because of their dependence on high relative humidity, dust mites are not thought to be relevant allergens in arid and semiarid regions. High-altitude areas are known to exhibit a lower prevalence of dust mites and a lower prevalence

of mite sensitivity in atopic patients.¹⁷⁻¹⁹ This difference is thought to be related to the lower humidity in such areas rather than the altitude per se. In dry climates, such as the desert southwest, Arlian¹⁰ found that the use of evaporative coolers during the summer months was associated with active mite populations. However, during the dry season, few if any active mites were seen. A previous study of dust mite prevalence in Colorado demonstrated a very small number of mites in native Denver homes.²⁰ Consequently, dust mite sensitivity has been considered to be of minimal clinical significance in the Colorado area.

Evaporative coolers, also known as "swamp coolers," are units that cool the air by adding moisture to it; conventional air conditioners dehumidify the air. Because of their cooling capacity, humidifying effect, and relative economic advantage, evaporative coolers are a popular form of air-conditioning in drier climates. Evaporative coolers can raise the indoor relative humidity, thereby creating a favorable environment for mite survival. The objective of this study was to determine the prevalence of *Dermatophagoides* allergen in Colorado homes in which evaporative coolers are used.

METHODS

Dust collection

Households that had been established in the Colorado Front Range area (altitude of approximately 5280 feet above sea level) for at least 5 years were recruited into the study. Nineteen homes in which central evaporative coolers were used were designated as the study group. Nineteen homes in which no form of air-conditioning was used comprised the control group. Samples of dust were obtained from all houses in May and again in late August 1993. Collection months were chosen to precede and follow the use of evaporative coolers during the summer months. The August collection time was also expected to coincide with the viable mite population peak.^{15, 16} Samples were collected by vacuuming 1 m² for 2 minutes in a standard manner onto a dust "trap filter" between the hose and the wand of a portable Dirt Devil vacuum cleaner (Royal Appliance Manufacturing Company, Cleveland, Ohio). The dust samples were sealed in plastic bags and stored at 4° C until they were extracted. Collection of dust samples was from five sites: bedroom mattress, carpeting adjacent to the bed, family room upholstered furniture, carpeting next to that furniture, and carpeting near the vent from the evaporative cooler. The samples in May and August were taken from the same sites.

Extraction of dust

The sieved, fine dust was extracted (1:20 wt/vol) by rotation of a 100 mg sample in 2 ml of 0.05 mol/L Tris (pH = 8.2) and 25% glycerin solution overnight at 4° C. For samples in which there was less than 100 mg of dust available, 50 mg was used with 1 ml of buffer. The samples were centrifuged and the supernatants were stored at -70° C until ELISA analysis was performed. Extraction methods differ in the literature, especially in the use and concentration of glycerin in the extracting solution.^{5, 6, 14, 15, 21-23} Therefore to validate the method used in this study, additional matched aliquots were reassayed with both the Tris/25% glycerin solution and a borate-buffered solution (BBS, pH = 8) with a room temperature extraction of 2 hours.^{14, 15} Fifteen positive samples and 38 negative samples were used for comparison of the extraction methods. The BBS extraction and Tris/25% glycerin extraction samples were assayed simultaneously.

Sandwich ELISA analysis for Der p 1 and Der f 1

Monospecific antibodies (Miles Allergy Products, Spokane, Wash.) were used in two-site enzyme immunoassays to quantitate Der p 1 and Der f 1.^{24, 25} Briefly, 96-well microtiter plates (Immulon IV; Dynatech Laboratories, Chantilly, Va.) were coated with monospecific antibodies to Der p 1 or Der f 1. Dust extracts were incubated on the plates for 2 hours at room temperature, then washed. Bound allergen was detected with a second enzyme-conjugated guinea pig antibody, which recognizes another epitope common to both Der p 1 and Der f 1 (Miles Allergy Products). After addition of the enzyme substrate system (alkaline phosphatase and para-nitrophenylphosphate), the quantitation of Der p 1 and Der f 1 was obtained by comparing the optical density at 405 nm (EL308; Bio-Tek Instruments, Winooski, Vt.) with the standard curve. The standard curves were constructed with a series of dilutions of mite extracts (Miles Allergy Products), ranging from 0.2 µg/gm dust to 300 µg/gm dust. The interassay variability was ±22%, and the intraassay variability was ±18%. All samples were run in duplicate. Results are reported as micrograms of Group I allergen per gram of dust. The monoclonal antibodies, enzyme-conjugated antibody, and standard mite extracts were provided as gifts by Miles Allergy Products.

Housing survey, temperature, and relative humidity

Information regarding the house, furnishings, humidifier use, and water damage was obtained at the time of each visit. Daily indoor temperature and relative humidity were recorded by the study participants for the duration of the study period with a Taylor Ashton Humidiguide hygrometer (no. 5502; Thermometer Corporation of America, Fletcher, N.C.). Participants were asked to record this data daily at the same time of day

TABLE I. Group I mite allergen in homes with evaporative coolers and control homes

Homes	May			August			
	No. of + homes (n = 19)	No. of + samples (n = 95)	Mean ± SEM (µg Ag/gm dust) (n = 95)	No. of + homes (n = 19)	No. of + samples (n = 95)	Mean ± SEM (µg Ag/gm dust) (n = 95)	
Study	1 (2%)	1 (1%)	0.08 ± 0.08 (0-7.9)	12 (63%)	48 (50.5%)	11.02 ± 1.52 (0-53.5)	p < 0.05*
Control	2 (10%)	2 (2%)	0.13 ± 0.10 (0-8.7)	5 (26%)	5 (5.3%)	0.19 ± 0.10 (0-7.7)	p = NS
	p = NS	p = NS	p = NS	p < 0.05*	p < 0.00001*	p < 0.0001†	

Plus sign denotes samples with concentrations equal to or greater than 2 µg allergen per gram of dust.

Ranges are given in parentheses.

Ag, Allergen; NS, not significant.

*Chi square analysis.

†Mann-Whitney U rank sum test.

(evening hours). The hygrometers were placed between 0.5 and 1.0 m above the ground in the main living area of the house. None of the hygrometers were placed directly in front of an outflow vent from the centralized evaporative cooling system. Participants were asked to record duration of evaporative cooler use as well.

Data analysis

Comparisons between the homes with evaporative coolers and the control homes were made by using nonparametric methods. The incidence of values of 2 µg/gm dust or greater (positive) and less than 2 µg/gm dust (negative) was compared by means of chi square analysis for differences between the study and control groups. Differences between homes with evaporative coolers and control homes and differences between May and August allergen concentrations were compared by using the Mann-Whitney U rank sum test. Associations among temperature, relative humidity, and mite allergen concentrations were evaluated with linear regression analysis. Differences between average relative humidities were analyzed with the Mann-Whitney test for nonparametric data. Significance was defined by a *p* value of less than 0.05. All statistical tests were performed with the Minitab statistical software for the MacIntosh computer (Minitab, Inc., State College, Pa.).

RESULTS

The relevant concentration of mite allergen used as a positive result was defined as 2 µg Group I allergen per gram dust or greater, the established critical threshold for sensitization.^{23, 26} Of the 38 homes studied, 17 had significant concentrations of mite allergen. Only one home in each group had positive concentrations when sampled in May. However, in August, 12 of 19 homes with evaporative coolers (63%) and 5 of 19 control homes

(26%) had mite allergen concentrations greater than 2 µg/gm dust. This was statistically significant for both May versus August and study versus control homes with *p* values of less than 0.05 as determined by chi square analysis (Table I). In the analysis of individual samples (rather than homes), only 3 of 190 samples in May had positive concentrations: one from a study home and the other two from a single control home (*p* = NS). From August data, 48 of 95 samples from homes with evaporative coolers (50.5%) and 5 of 95 samples from control homes (5.2%) had positive concentrations with a *p* value of less than 0.00001 as determined by chi square analysis (Table I).

The range of Group I allergen Der p 1 + Der f 1 in the study group was from 0 to 53.5 ± 14.8 µg/gm dust. The mean concentration of Group I allergen in the study group in August was 11.2 µg/gm dust ± 1.52 SEM—a level above the established threshold of 10 µg/gm dust for symptomatic exposure.^{23, 26} The range for the control group samples was from 0 to 8.7 ± 0.98 µg/gm dust. The mean for the control group in August was 0.19 µg/gm dust ± 0.10 SEM (Table I, Fig. 1). Der f 1 was the only allergen detected in the positive control home samples and in the May samples of both control and study homes.

Dust mite allergen was detected in samples from all types of sites, with the highest concentration of allergen occurring in the mattresses and family room furniture. In general, carpet samples were only positive if the corresponding furniture was positive as well. Three of the five positive control home samples were obtained from furniture that had been imported to Colorado from mite-preva-

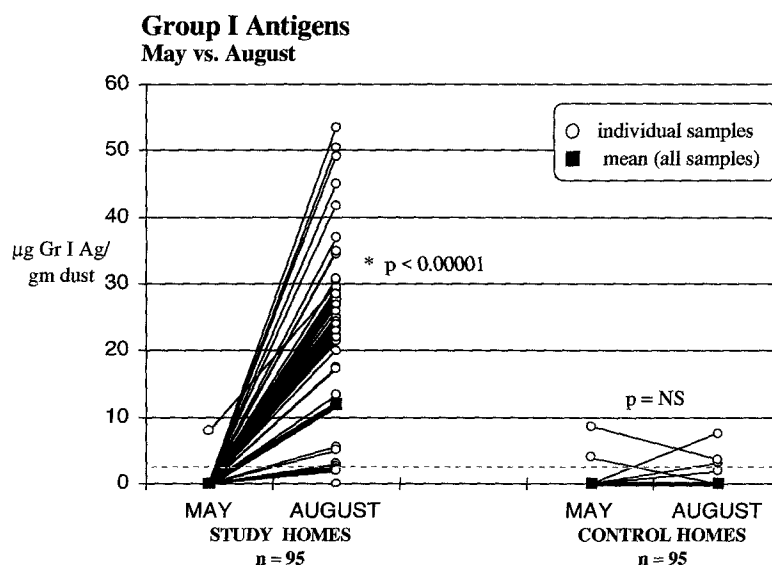


FIG. 1. Results of measurement of Group I allergen concentrations in dust samples from study and control homes sampled in May and August. Group I allergen (Der p 1 + Der f 1) was measured by ELISA and is expressed in micrograms of allergen per gram of dust. *Chi square test.

TABLE II. Average relative humidity in homes with evaporative coolers ($n = 19$) and control homes ($n = 19$)

Homes	May		August		
	Mean RH (%)	Range (%)	Mean RH (%)	Range (%)	
Study	51.1	36-72	59.1	47-78%	$p = 0.006^*$
Control	41.3	33-49	43.1	31-55%	$p = NS$
	$p < 0.001^*$		$p < 0.001^*$		

RH, Relative humidity; NS, not significant.

*Mann-Whitney U test.

lent areas; one of these samples was from a sofa that had been in Colorado for 19 years.

Relative humidity and temperature were averaged for each home over each month of the study. The homes with evaporative coolers had average relative humidities that were greater than 50% in both May and August, whereas the control homes had average relative humidities below 45%. The differences noted between relative humidities in study and control homes were statistically significant ($p < 0.001$, Mann-Whitney test). The difference in relative humidity from May to August within the study group was also significant ($p = 0.006$, Mann-Whitney test), whereas May versus August relative humidity in the control group was not significantly different (Table II).

Regression analysis between relative humidity and Group I allergens showed a positive correlation ($r = 0.47$ for Der p 1; $r = 0.51$ for Der f 1; $p <$

0.001) in August. The relationship between relative humidity and mite allergen levels reveals a threshold at 54%. Below this level of humidity, only 5 of 53 positive samples were detected: one *D. pteronyssinus* and four *D. farinae*, in the range of 2 to 8 $\mu\text{g/gm}$ dust. Some homes with greater than 54% relative humidity did not demonstrate significant allergen, and a few homes showed the presence of mite allergen despite relative humidities as low as 32% for *D. farinae* and 42% for *D. pteronyssinus* (Fig. 2). No significant correlation or threshold pattern with temperature was noted. No direct correlation was noted when housing survey variables and mite concentration were analyzed. In the evaporative cooler group, 11 of 19 residents reported the use of room or central humidifiers during the winter months, whereas only 6 of 19 residents of control homes used humidifiers.

Comparison of extraction methods revealed that

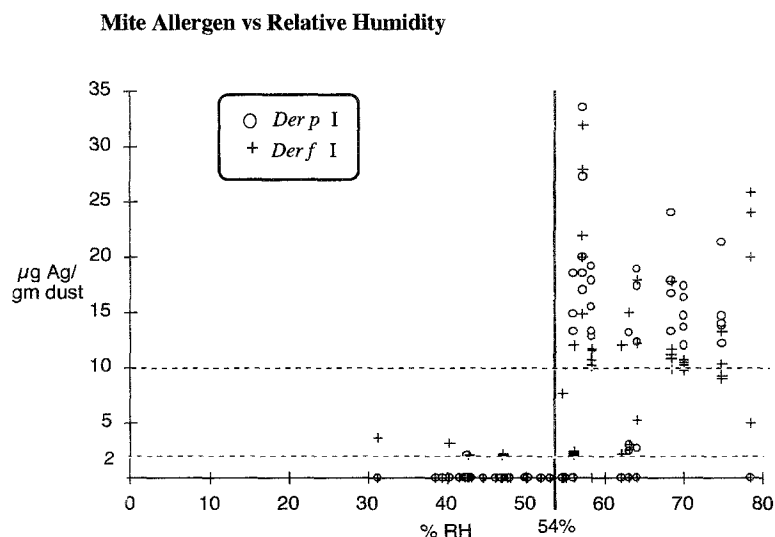


FIG. 2. Relationship between concentration of mite allergen (Ag) in dust samples and average relative humidity (RH) in August.

all samples, which were positive initially, were again positive as determined by both extraction methods. The differences between mite concentrations when both methods were compared simultaneously was not statistically significant according to analysis of variance for nonparametric data. Furthermore, no consistent tendency for one method to demonstrate higher or lower concentrations than the other was observed. Two of 38 initially negative samples were positive when extracted with BBS but remained negative when extracted with Tris/25% glycerin. This was not statistically significant.

DISCUSSION

Measurement of Group I allergen in dust samples from Colorado homes demonstrates that significant amounts of mite allergen can be found in semiarid climates. Although the previous study done in Denver reported a low prevalence of dust mites, the study population did not include homes with evaporative coolers.²⁰ The ability of evaporative coolers to significantly increase the indoor relative humidity provides a favorable environment in which dust mites can survive. In this study homes in which evaporative coolers were used demonstrated higher relative humidities than the control homes, particularly in August after peak evaporative cooler use. On the other hand, control homes consistently demonstrated average relative humidities less than 45%, reflecting the semiarid climate of the Denver area. The higher average relative humidity in study homes in May, a time

when the evaporative coolers were not being used, may be related to the use of humidifiers during the winter months. Although temperature and relative humidity readings were not measured during the winter months, the presence or absence of humidifiers was assessed by questionnaire. The home owners who had evaporative coolers used humidifiers (either room or central) more frequently than the control group: 11 of 19 compared with 6 of 19.

Detection of Der f 1 alone in control home samples from furniture that had been imported to the Denver area was noted. The ability to detect Der f 1 even after the furniture had been in Colorado for 19 years is of particular interest given *D. farinae*'s ability to withstand harsher conditions. Moyer and Nelson²⁰ examined dust samples from imported furniture and noted the presence of live mites after 2 years; however, longer time intervals were not examined in that study. Therefore it is not known how long mites are able to survive in semiarid environments when imported from mite-prevalent areas. The stages of mite development, which include several periods of ecdysis, may allow for a dormant existence that is resistant to unfavorable microenvironments for extended periods. Specifically, when dust mites are in the prolonged protonymph stage, they have significantly reduced metabolic rates and are extremely resistant to desiccation. The return of favorable conditions (higher relative humidity) could spur the transition to an active development stage, allowing the mites to proliferate.^{10, 11}

An alternate theory is that imported mites died

long ago but the allergen remained and was removed with sampling. Although live mite count data may have helped clarify this, resources were limited, especially regarding our expertise in mite species identification and differentiation. Also, the absence of mite allergen in May samples followed by the detection of allergen in August samples (obtained from the same site) does not support this alternate theory.

The central importance of dust mite exposure to the development of asthma has been well demonstrated in the literature.* Several reports have validated the concept of sensitizing and symptom-provoking levels of mite allergen at 2 and 10 $\mu\text{g/gm}$ dust, respectively.^{23, 26} These reports have not consistently used the same extraction procedures; however, extraction without glycerin is common. It has been suggested that higher concentrations of glycerin may augment the level of extracted mite allergen, while also interfering with the assay (Platts-Mills, personal communication). To assure that the mite concentrations reported here were both reproducible and comparable to those in the literature, aliquots of a number of the dust samples were divided and extracted with both Tris/glycerin and with BBS alone, replicating a commonly used procedure. Results were reproducible both within and between extraction methods: no differences or trends were noted. Therefore the implications of sensitizing concentrations discussed in the literature may be applied to the mite concentrations found in this study.

This study was not designed to evaluate clinical mite allergy; no skin testing or symptom correlation was performed, nor were the homes sampled necessarily those of atopic individuals. The prevalence of skin test positivity to dust mite in native Coloradans is not known. Many Colorado Front Range allergists have not routinely tested for sensitivity to dust mites. In addition, the population of Coloradans who have not traveled to mite-prevalent areas and therefore have minimal risk of sensitization) has not been established.

A relative humidity of at least 54% was needed to detect significant concentrations of allergen. This is similar to the minimum amount of relative humidity needed to demonstrate mite allergen reported in the literature. However, there were homes with greater than 54% relative humidity that did not demonstrate significant allergen levels and a few that showed the presence of mite

allergen despite relative humidities as low as 32% for *D. farinae* and 42% for *D. pteronyssinus* (Fig. 2). The wide variation of mite allergen concentrations noted in the homes with evaporative coolers is not distinguished solely by the indoor relative humidity measurements. The difference in allergen concentrations in these homes suggests that other features of the home, which were not measured, may have an impact on the dust mite microenvironment. Such conditions within the dust mite milieu may be critical to mite survival and growth.

The results reported in this study demonstrate that alteration of the indoor environment can influence the survival and proliferation of dust mites. The use of evaporative coolers, which increase indoor relative humidity, clearly facilitates the survival and growth of dust mites in an otherwise unfavorable setting. Therefore one should closely examine any factors within the home that could support the presence of a specific allergen and not simply assume that an allergen is insignificant on the basis of regional climate.

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