

Inhaled Aeroallergen and Storage Mite Reactivity in a Wisconsin Farmer Nested Case-control Study

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A nested case-control study was undertaken to assess the role of antibody (Ab) to a panel of antigens associated with hypersensitivity pneumonitis in the development of lung disease among Wisconsin dairy farmers. Immunologic reactivity to a panel of inhaled allergens was assessed in the Ab⁺ cases and compared to the Ab⁺ control, Ab⁻ control, and Ab⁻ blue-collar control subjects by skin tests, RAST assays, and a modified American Thoracic Society questionnaire. The most prevalent allergens among the cases as determined by either skin test or RAST assays were the house dust mites (HDM) (21.6%), storage mites (11.2%), grain smuts (11.2%), *Cladosporium* (7.5%), *Aspergillus* (6.0%), and cattle (5.2%). No other allergens appeared to be different from in nonfarming populations. Of the storage mite reactors, IgE to *Lepidoglyphus destructor* (7 of 8) was the most frequently found, followed by *Tyrophagus putrescentiae* (6 of 8), *Glycyphagus domesticus* (5 of 8), *Chortoglyphus arcuatus* (5 of 8), and *Acarus siro* (2 of 8). *L. destructor* was also isolated from the farm of one of the cases. There was no difference in the total IgE levels among the groups. Reactions to HDM, storage mites, and grain smuts were significantly more frequent among the cases ($p < 0.05$). We conclude from this study that there is a significant increase in reactivity to certain inhaled allergens among those dairy farmers reporting barn-associated respiratory symptoms that is unrelated to past exposure to the causative agents of farmer's lung disease.

A variety of environmental respiratory illnesses afflict the farming population. Much attention has been given to the causes and ecologic treatment of hypersensitivity pneumonitis in farmers (1, 2). Nonimmunologic responses have also been described by Emanuel and coworkers (3). The cause and avoidance treatment of atopic respiratory diseases in farmers have not received much attention in the United States. Studies from the British Isles have shown that the prevalence of atopic disease in Orkney farming families was 17.5% and 30% in Scottish farm workers (4, 5). The study of Scottish farm workers showed that the majority of atopic individuals were sensitive to storage mite extracts and hay dust. Different mite species present in large quantities in these environments have been identified (6, 7). Recently, Campbell and coworkers (8) used an immunochemical technique to determine the quantity of specific atmospheric allergens present in barn air collected from barns in Upstate New York and Rochester, Minnesota. Their methods identified the allergens of *Aspergillus fumigatus* and the storage mite *Lepidoglyphus destructor* as the most prevalent aeroallergens. In the past decade, a number of studies have defined the importance of storage mite allergens, the ecologic factors favoring their growth, and the relative cross-reactivity of the different mite allergens (9–12). Most of the work has been done

in European farming communities. The current study attempts to demonstrate whether the storage mite and other barn-associated allergens are important in the development of respiratory disease among central Wisconsin dairy farmers. Using a nested case-control design, we evaluated the presence of skin test and/or RAST activity to a panel of aeroallergens. The panel of allergens included those unique to the farm as well as the routine allergens used to screen the general patient population from the area.

METHODS

Patient Populations

The original description of the population studied in 1977 was by Gruchow and coworkers (13) from a three-county area in central Wisconsin. A 33% random sample of the registered farms defined the basis of a farming population of 1,511 subjects studied. This population served as the basis for the current study. A resurvey by mail and telephone was conducted in 1987 with a 72.5% response rate. Antibody (Ab) reactivity to a panel of thermophilic actinomycetes and fungal organisms associated with the hypersensitivity pneumonitis was used to segregate the subjects. This was the same panel used for the 1977, survey as previously described (13), using a previously published enzyme immunoassay (14). All subjects with demonstrable antibody were asked to participate in the current study. A population matched for age, sex, and smoking history was used as referent or control groups for this case-control study. The institutional review board approved this study before the original population definition and again before these more recent studies.

The cases were defined based on responses to a nurse-administered questionnaire. A questionnaire standardized by the American Thoracic Society for the detection of respiratory disease was extended to determine the relationship of farming exposure to the clinical history or symptoms. Specifically, the patients were queried for a history of wheezing, chest tightness, cough, or fever associated with the barn or silo. Other criteria used to group subjects included recurrence of symptoms follow-

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TABLE 1
CASE DEFINITION

Positive Responses to the Following Questions Classify a Patient as a Case*	Yes/No
If not disabled from walking by any condition other than heart or lung disease, are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? If yes, do you have to walk slower than people of your own age on level ground because of breathlessness? Odds ratio (OR)	
Have you ever been sick after uncapping a silo? Symptoms: (a) cough, (b) fever, (c) chest tightness, (d) breathlessness OR	
Have you ever been sick after handling hay or moldy grain (other than moldy silage)? Symptoms: (a) cough, (b) fever, (c) chest tightness, (d) breathlessness OR	
Does your chest ever sound wheezy or whistling during or following work (up to 8 h following) in your barn or silo? OR	
Does your chest ever feel tight during or following work (up to 8 h following) in your barn or silo? OR	
After you have been away from your farm for 2 days or more, on resumption of farming, have you noted any (or increased) respiratory symptoms (include coughing, wheezing, chest tightness, breathlessness, fever, or fatigue)?	

* Serologic response to the thermophilic actinomycete panel stratifies a patient at Ab⁺ or Ab⁻ cases.

ing absence from farming and a subjective comparison of their breathlessness compared with that of their peers.

Case groups included those patients with positive response to the clinical questions (table 1). The case groups were divided into those with and without Ab⁺ status. Farming control groups included those subjects from the population without positive clinical responses on the questionnaire. Subjects were matched for age, sex, smoking history, and antibody status.

A blue-collar control group consisted of individuals without a history of living or working on a farm in the past 5 yr and whose occupational environment was not considered dusty. Postal workers, medical center building service personnel, and outreach drivers were recruited and subjected to the same clinical and laboratory protocol as the farmers.

The field survey consisted of pulmonary function analysis using a Spirotech[®] spirometer (Anderson Instruments, Atlanta, GA), questionnaire,

and serology. The clinical protocol consisted of a physical examination, routine laboratory screens, repeat serology, posteroanterior and lateral chest X-ray, methacholine challenge, and complete pulmonary function testing, skin tests, and bronchoalveolar lavage. Although a complete medical history was taken, emphasis was placed on the definition of respiratory disease.

Skin Testing

Skin tests were performed on the upper outer arm by the standard intradermal technique using 1:500 wt/vol standardized commercially prepared extracts. Dander, pollens, and molds were obtained from Greer Laboratories (Lenoir, NC) and mite extracts from Berkeley Biologics (Berkeley, CA). Skin tests were performed before the methacholine challenge. Controls included histamine, codeine, and diluent. The reactions were read after 15 min by measuring the largest wheal diameter and the largest diameter of the erythema. A skin test was considered positive if the resultant wheal was greater than 5 mm more than the negative control and had erythema. The following allergens were used for testing: dander included cat, dog, hog, and cattle; pollens included ragweed, mixed grasses, birch, maple, and oak; molds included *Alternaria tenuis*, *Aspergillus* mix, *Penicillium* mix, *Cladosporium herbarum*, *Hormodendrum hordei*, *Helminthosporium sativum*, grain smut mix; house dust mites (HDM) included *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus*.

RAST Assays

RAST assays were used to determine the presence of IgE antibody to the storage mites, including *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, and *Chortoglyphus arcuatus*. RAST assays were purchased from Pharmacia Diagnostics (Fairfield, NJ) and performed according to the manufacturer's recommendations. A positive was considered greater than 1⁺ reactivity. IgE antibodies to *C. arcuatus* was kindly assayed by Dr. Enrique Fernandez-Caldas, University of South Florida, and a positive was defined as greater than 1.0% total counts bound.

Statistical Analysis

Statistical analysis was performed using the standard chi-square analysis of contingency tables or Fisher's exact test if cells contained fewer than five events. Chi-square values and p values for the test for homogeneity were calculated using the statistical software program EpiInfo[®] (Centers for Disease Control, Atlanta, GA). Fisher's exact tests were performed in r × c contingency tables as described by Mehta and Patel (15).

RESULTS

Comparing the demographics of the groups, there was no significant difference in age, sex, race, education, smoking history, or

TABLE 2
RESPIRATORY DISEASE AMONG WISCONSIN DAIRY FARMERS:
CASE-CONTROL STUDY

	Ab ⁺ Case (n = 71)	Ab ⁻ Case (n = 11)	Ab ⁺ Control (n = 22)	Ab ⁻ Control (n = 16)	Nonfarming Control (n = 14)
Mean age, yr	51.7	47.2	49.6	50	50.6
White, %	100	100	100	75.0	100
Male, %	88.7	90.9	77.2	81.2	78.6
High school education, %	70.4	72.7	91	68.7	92.8
Never smoked cigarettes, %	67.6	54.5	68.2	68.8	64.3
Currently smokes cigarettes, %	7.0	18.0	4.6	6.3	7.0
Dairy farming, %	70.4	63.6	72.7	56.2	
Currently feeding or milking cows (dairy or beef), %	71.8	54.5	77.3	50	
Years farming, mean	32.8	28.2	31.8	28.6	
Total acres, mean	315	270	233	199	
Ever used respiratory in farm work, %	63.3*	27.3	36.3	43.7	

* p Value = 0.022; all other values > 0.05.

TABLE 3
FREQUENCY OF REACTIVITY TO A PANEL OF AEROALLERGENS
IN CENTRAL WISCONSIN FARMERS

	Ab ⁺ Case (n = 71)	Ab ⁻ Case (n = 11)	Ab ⁺ Control (n = 22)	Ab ⁻ Control (n = 16)	Nonfarming Control (n = 14)
Dander (any)	15.5*	9.1	4.5	6.2	0
Cat	5.6	0	0	6.2	0
Dog	1.4	0	0	6.2	0
Cattle	9.9	9.1	0	6.2	0
Hog	1.4	0	4.5	6.2	0
Pollens (any)	7.0	0	18.2	0	7.1
Grass	1.4	0	13.6	0	0
Ragweed	2.8	0	4.5	0	7.1
Birch	2.8	0	4.5	0	0
Oak	2.8	0	4.5	0	0
Maple	1.4	0	9.1	0	7.1
Molds (any)	36.6	27.3	4.5	25.0	0
<i>A. fumigatus</i>	11.3	18.2	4.5	6.2	0
<i>Penicillium</i> sp.	9.9	9.1	0	6.2	0
<i>Helminthosporium</i>	7.0	9.1	0	18.7	0
<i>Hormodendrum</i>	8.5	0	0	6.2	0
<i>Cladosporium</i>	14.1	9.1	0	6.2	0
<i>Alternaria tenuis</i>	2.8	9.1	0	6.2	0
Grain smut	21.1	18.2	0	18.7	0
House dust mites	40.1	9.1	27.3	25.0	14.3
<i>D. pteronyssinus</i>	38.0	9.1	18.2	25.0	14.3
<i>D. farinae</i>	40.1	9.1	27.3	25.0	14.3
Storage mites (RAST)	9.9	9.1	0	6.2	0
<i>Acarus siro</i>	2.8	0	0	6.2	0
<i>Lepidoglyphus destructor</i>	9.9	9.1	0	6.2	0
<i>Tyrophagus putrescentiae</i>	8.5	0	0	6.2	0
<i>Glycyphagus domesticus</i>	7.0	0	0	6.2	0
<i>Chortoglyphus arcuatus</i>	5.6	9.1	0	6.2	0
Any aeroallergen	70.4	36.4	31.8	43.7	14.3

* Percentage of subjects with skin test > 5 mm wheal or RAST ≥ 2+.

parameters of farming exposure (table 2). There was a higher rate of farmers who used respirators among the Ab⁺ cases than the other referent groups. Whether this was related to awareness of respiratory dangers of exposure or preexisting respiratory disease was not determined.

The frequency of skin test and RAST reactivity to the panel of aeroallergens tested is presented in table 3 for the farming groups and the nonfarming control group. The most frequent aeroallergen reactions were seen with HDM, grain smuts, molds, storage mites, and cattle. The nonfarming control subjects reacted primarily to HDM and pollens.

The statistical comparison of the responses among the groups is outlined in tables 4, 5, and 6. There was a highly significant difference between the farmers and the nonfarmers in overall reactivity. When reactivity to individual allergens was evaluated statistically, only the reactivity of the mold and smut group showed significance between farmers and nonfarmers. The farming groups are compared in table 5. Aeroallergen reactivity to the panel was higher in the case groups, particularly the Ab⁺ cases, than the farming control groups. There was no difference between the Ab⁻ and Ab⁺ groups.

The criteria for case definition included, among other parameters, a reported wheezing and/or chest tightness in the barn or silo. There was no significant difference in reactivity to an allergen between those farmers who reported these symptoms and those who did not (table 6).

DISCUSSION

This study was a comparison of the reactivity to aeroallergens as measured by either skin tests or RAST assays in a nested case-control study of central Wisconsin farmers. The cases were defined by parameters useful in segregating farmers based on their past exposure to microorganisms associated with hypersensitivity pneumonitis and their symptoms associated with the barn environment. Based on the inability to show a difference between the Ab⁺ and the Ab⁻ groups, the association of the exposures to these microorganisms and the development of aeroallergen reactivity are probably unrelated.

TABLE 4
SKIN TEST AND RAST REACTIVITY AMONG
FARMERS AND NONFARMERS

	Any Determinant	Dander	Pollen	Molds, Smuts	House Mite	Storage Mite
Farmer groups (n = 120)	68*	14	9	34	40	9
Nonfarmers (n = 14)	2	0	1	0	2	0
p Value	0.006	0.36	1.00	0.02	0.22	0.60
Odds ratio	7.85	—	1.05	—	3.00	—

* Number of patients with skin test > 5 mm wheal or RAST ≥ 2+.

TABLE 5
SKIN TEST OR RAST REACTIVITY AMONG FARMING CASE AND REFERENT GROUPS

	Any Determinant	Dander	Pollens	Molds, Smuts	HDM	Storage Mites
Ab ⁺ cases (n = 71)	50	11	5	26	29	7
Ab ⁻ cases (n = 11)	4	1	0	3	1	1
Ab ⁺ controls (n = 22)	7	1	4	1	6	0
Ab ⁻ controls (n = 16)	7	1	0	4	4	1
p Values (OR)						
Ab ⁺ cases versus all controls	0.003	0.45	0.12	0.035	0.13	0.49
Cases versus controls	0.005 (3.31*)	0.22 (3.09)	0.46 (0.55)	0.022 (3.61*)	0.37 (1.62)	0.27 (4.00)
Ab ⁺ versus Ab ⁻	0.09 (2.3)	0.73 (1.85)	0.20 (-)	0.94 (1.17)	0.10 (2.66)	1.00 (1.02)

* Odds ratio is significantly different than 1.00.

TABLE 6
SKIN TEST AND RAST REACTIVITY AMONG FARMERS REPORTING
WHEEZING OR CHEST TIGHTNESS IN THE BARN

	Any Determinant	Dander	Pollens	Molds, Smuts	HDM	Storage Mites
Wheezing and/or chest tightness in the barn (n = 33)	60.6*	15.1	9.1	39.4	36.4	12.1
No reported symptoms (n = 64)	46.9	10.9	6.2	20.3	35.9	7.8
p Value	0.28†	0.53	0.68	0.07	0.85	0.55

* Percentage of total.

† As determined by chi-square test.

The farmers in general showed a considerably greater reactivity to the aeroallergen panel than the nonfarmers. The allergens to which the farmers reacted seemed to be those that may be unique to the barn environment. The number of nonfarmers in this study was limited. This probably accounts for the inability to show a statistical significance in the individual allergens. It should be noted that the aeroallergens that were tested included those unique allergens as well as allergens commonly represented in the central Wisconsin patient population (16).

The nested case-control study demonstrated a relationship between the cases and aeroallergen reactivity compared to the farming control groups. Since this population was selected on the basis of respiratory reactivity to the barn or silo environment, it is not surprising to find more respiratory disease among this group. It was interesting, however, that the respiratory disease was not limited to the hypersensitivity pneumonitis that was originally targeted. The IgE-mediated sensitivity appears to be a significant problem among these workers, as measured by skin test or RAST. Reported chest symptoms may not be a good indicator of allergic disease, since we saw no significant difference between those who reported symptoms and those who did not. It should be noted that we screened only for barn- or silo-associated chest symptoms. Those patients with symptoms from other environmental sources were not segregated, nor were patients with symptoms of allergic rhinoconjunctivitis. Previous studies by our group reported no significant difference in pulmonary function accountable by antibody status or smoking (17). However, there was an association of reported chronic symptoms of bronchitis and occupational asthma in the Ab⁺ cohort (2). In the present nested case-control study there were no significant functional abnormalities in lung function and airway reactivity among Wisconsin dairy farmers who are Ab⁺ and relate a history of barn-associated symptoms (18).

Which etiologic agents are particularly responsible for the atopic sensitization in farmers remains to be seen. It seems clear that certain allergens, like the cattle dander, grain smuts

and other molds, and the storage mites, are likely to be involved because of the opportunities for exposure. The high rates of reactivity to the HDM are not surprising because this is the most prevalent positive skin test in our allergic rhinitis and asthma patient population (16). The rates of storage mite reactivity appear to be high compared to those in nonfarming control subjects. In fact, our experience in the laboratory suggests that these allergens are somewhat unique to the farming population.

These are probably not reactions that result from cross sensitivity with the more common HDM. We successfully isolated at least two of the named species from forage samples from farms in this study. Immunologic studies have not shown a great deal of cross allergenicity between storage mites and HDM (19).

In conclusion, we were able to show that the farmers in our study had a higher level of atopic sensitivity than nonfarming control subjects. This is not dependent upon antibody positivity to a panel of antigens responsible for hypersensitivity pneumonitis. Sensitivity to molds, grain smuts, and storage mites were especially significant among farmers. Epidemiologic studies of farming populations should bear these findings in mind and screen for signs and symptoms of allergic rhinoconjunctivitis.

References

- Grant IW, Blyth W, Wardrop VE, Gordon RM, Pearson JC, Mair A. Prevalence of farmer's lung in Scotland: a pilot survey. *Br Med J* 1972; 1:530-4.
- Marx JJ Jr, Guernsey J, Emanuel DA, Merchant JA, Morgan DP, Kryda M. Cohort studies of immunologic lung disease among Wisconsin dairy farmers. *Am J Ind Med* 1990; 18:263-8.
- Emanuel DA, Wenzel FJ, Lawton BR. Pulmonary mycotoxicosis. *Chest* 1975; 67:293-7.
- Cuthbert OD. The incidence and causative factors of atopic asthma and rhinitis in an Orkney farming community. *Clin Allergy* 1981; 11:217-25.
- Cuthbert OD, Jeffrey IG, McNeill HB, Wood J, Topping MD. Barn allergy among Scottish farmers. *Clin Allergy* 1984; 14:197-206.
- Leskinen L, Klen T. Storage mites in the work environment of farmers. *Eur J Respir Dis* 1987; 152(Suppl:101-11).
- Terho EO, Leskinen L, Husman K, Karenlampi L. Occurrence of storage mites in Finnish farming environments. *Allergy* 1982; 37:15-9.
- Campbell AR, Swanson MC, Fernandez-Caldas E, Reed CE, May JJ, Pratt DS. Aeroallergens in dairy barns near Cooperstown, New York and Rochester, Minnesota. *Am Rev Respir Dis* 1989; 140:317-20.

9. Cuthbert OD. Storage mite allergy. *Clin Rev Allergy* 1990;8:69-85.
10. Hart BJ, Whitehead L. Ecology of dust mites in Oxfordshire. *Clin Exp Allergy* 1990; 20:203-9.
11. Korsgaard J, Dahl R, Iversen M, Hallas T. Storage mites as a cause of bronchial asthma in Denmark. *Allergol Immunopathol* 1985; 13:143-9.
12. Van Hage-Hamsten M, Johansson SGO. Clinical significance and allergenic cross-reactivity of *Euroglyphus maynei* and other nonpyroglyphid and pyroglyphid mites. *J Allergy Clin Immunol* 1989; 83:581-9.
13. Gruchow JW, Hoffmann RG, Marx JJ Jr, Emanuel DA, Rimm AA. Precipitating antibodies to farmer's lung antigens in a Wisconsin farming population. *Am Rev Respir Dis* 1981; 124:411-5.
14. Marx JJ Jr, Gray RL. Comparison of the enzyme-linked immunosorbent assay and double immunodiffusion test for the detection and quantitation of antibodies in farmer's lung disease. *J Allergy Clin Immunol* 1982; 70:109-13.
15. Mehta CR, Patel NR. A network algorithm for performing Fisher's exact test in $r \times c$ contingency tables. *J Am Stat Assoc* 1983; 78:427-34.
16. Twigg JT, Allen R. The house dust mite identified as the principal indoor allergen in a Wisconsin pediatric allergic rhinitis/asthma population. *Immunol Allergy Pract* 1989; 11:255-8.
17. Guernsey JR, Morgan DP, Marx JJ, Horvath EP, Pierce WE, Merchant JA. Respiratory disease risk relative to farmer's lung disease antibody status. In: Dosman JA, Cockcroft DW, eds. *Principles of health and safety in agriculture*. Boca Raton, FL: CRC Press, 1989; 81-4.
18. Merchant J, Miller E, Campbell J, Twigg J, Marx J, Ault B, Burmeister L. Case control assessment of lung function among dairy farmers (abstract). *Am Rev Respir Dis* 1991; 143(Suppl:A101).
19. Artian LG, Geis DP, Vyszynski-Moher DL, Bernstein IL, Gallagher JS. Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 1984; 74:166-71.