In Africa, there seems to be an inconsistent relationship between asthma (both self-reported and physician diagnosed) and aeroallergen sensitization, with subjects in urban areas demonstrating the relationship and subjects in rural areas demonstrating less association[4] or even total dissociation between aeroallergen sensitization and allergic diseases.[5] Worm infestation has been postulated as one of the factors responsible for this, perhaps by nonspecifically increasing IgE production[6] without changing the predisposition to allergic diseases. As a "side effect" of the nonspecific IgE production, subjects may be protected from allergic diseases by blocking cross-linking of other allergen-specific IgE[7] by parasite-induced raised total IgE, causing low allergic reactivity (and possible negative skin test results) despite a high degree of sensitization to those allergens. In this urban African cohort, total IgE is markedly correlated with *Ascaris* IgE.[1]

However, this cohort with low-level parasite infestation showed *Ascaris* sensitization to be strongly positively associated with aeroallergen sensitization. There was a dose-response relationship between increasing class of *Ascaris* IgE and sensitization to 1 or more allergen, sensitization to HDM and grass, and number of positive SPT results. Any sensitization to *Ascaris* was associated with more than double the prevalence of HDM sensitization and almost 4 times the prevalence of grass sensitization, and higher levels of *Ascaris* IgE were seen in those with HDM and grass sensitization. This may suggest that subjects in Africa with a genetic propensity to aeroallergen sensitization also have upregulation of their defense system against parasitic infection. Some comparisons, however, are based on rather small sample sizes, and the results should be confirmed in a larger trial. An alternative explanation for the association between *Ascaris* IgE and skin sensitization is confounding for other exposures such as cross-reactive allergens, which include HDMs.[8] One solution to this problem is to use component allergens from *Ascaris* that have no cross-reaction with invertebrate allergens as measures of *Ascaris* sensitization such as ABA-1 (Asc s 1).

Although having symptoms of asthma was associated with a higher probability of AHR, only 40% of the subjects who reported symptoms of asthma had a positive methacholine challenge. Limitations in the definition of both these classifications, namely, methacholine challenge via handheld spirometer rather than dosimeter and asthma by self-reported symptoms rather than physician diagnosis, may affect the correlation between them as well as with other variables. The lack of association between *Ascaris* sensitization and allergic diseases may also be ascribed to the limited sample size.

Studies in the same genetic group of subjects with active *Ascaris* infection found no association between infection and risk of aeroallergen sensitization or titers of specific IgE but did find a significant association with exercise-induced bronchospasm and a decreased risk of positive skin test results.[9] The study concluded that *Ascaris* might induce nonspecific inflammation resulting from *Ascaris* passage through the lungs, independent of an effect on IgE production. Although we measured *Ascaris* sensitization rather than active infection, the association with *Ascaris* sensitization and AHR could result from the same phenomenon, but this would not explain the marked association with aeroallergen sensitization.

However, the finding that *Ascaris* sensitization is strongly associated with AHR but not with asthma, eczema, or rhinitis and is dependent on the level of *Ascaris*-specific IgE in a dose-dependent fashion may suggest that other factors in the environment are protective against the development of allergic disease, despite the presence of allergen sensitization.

Michael Levin, MBChB, FCPaed (SA), MMed (Paed), Dip Allerg (SA), PhD[a]
Rudzani Muloiwa, MBChB, DCH (SA), FCPaed (SA), MSc (LSHTM)[b]
Peter Le Souëf, MBBS, FRACP, MD[c]
Cassim Motala, MBChB, FCPaed (SA), FACAAI, FAAAAI[a,†]

From [a]the Division of Allergy and [b]Ambulatory and Emergency Paediatrics, School of Child and Adolescent Health, Red Cross War Memorial Children's Hospital, Cape Town, South Africa; and [c]the School of Paediatrics and Child Health, University of Western Australia, Perth, Australia. E-mail: michael.levin@uct.ac.za.

†Deceased.

Supported by funds from ALLSA/GlaxoSmithKline. Jack Larsen (Thermo Fisher, formerly Pharmacia) donated skin and ImmunoCAP reagents, and Marilyn Koumbari (ACIC Ltd/Methapharm) donated methacholine.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Levin ME, Le Souëf PN, Motala C. Total IgE in urban Black South African teenagers: the influence of atopy and helminth infection. Pediatr Allergy Immunol 2008;19:449-54.
2. Levin ME, Muloiwa R, Motala C. Associations between asthma and bronchial hyper-responsiveness with allergy and atopy phenotypes in urban black South African teenagers. S Afr Med J 2011;101:472-6.
3. Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial hyperresponsiveness. Thorax 1983;38:760-5.
4. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. Am J Respir Crit Care Med 2003;168:313-7.
5. Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E, Britton J. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. Lancet 1997;350:85-90.
6. Pritchard D. Immunity to helminthes: is too much IgE parasite rather than host protective? Parasite Immunol 1993;15:5-9.
7. Godfrey R, Gradidge CF. Allergic sensitization of human lung fragments prevented by saturation of IgE binding sites. Nature 1976;259:484-5.
8. Acevedo N, Sanchez J, Erler A, Mercado D, Briza P, Kennedy M, et al. IgE cross-reactivity between ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009;64:1635-43.
9. Calvert J, Burney P. Ascaris, atopy, and exercise-induced bronchoconstriction in rural and urban South African children. J Allergy Clin Immunol 2010; 125:100-5.

Available online May 2, 2012.
doi:10.1016/j.jaci.2012.03.033

Fungal and atopic sensitization are low among farmers in the Agricultural Health Study

To the Editor:

Farm work may result in exposure to microbial bioaerosols. In agriculture, average airborne concentrations of fungal conidia are several orders of magnitude higher than in nonagricultural, indoor environments without water damage.[1] We used data from Iowa and North Carolina farmers to explore associations between farming activities and fungal sensitization.

We analyzed serum samples from a neurobehavioral substudy of 677 male private pesticide applicators in the Agricultural Health Study (AHS).[2] Blood was collected in 2006-2008 from male pesticide applicators who had completed all AHS interviews. Blood samples were not available for the whole cohort. Individuals were excluded if they had never been a farmer or had neurologic diseases; women were excluded because of their

TABLE I. Farming characteristics and fungal sensitization and atopy among 677 farmers in the AHS

	Fungal sensitized							Atopic (n = 14)*		Nonatopic (n = 536)				
	Yes (n = 28)		No (n = 649)		Odds							Odds		
Enrollment variables	n	Percent	n	Percent	ratio*	95% CI		n	Percent	n	Percent	ratio†	95% CI	
Grew up on a farm														
No	3	11	68	11				15	11	56	11			
Yes	25	89	575	89	1.0	0.3	5.5	126	89	474	89	1.1	0.6	2.2
Years worked/lived on farm														
<30	4	15	151	24				24	17	131	25			
≥30	23	85	489	76	1.9	0.6	7.7	114	83	398	75	1.8	1.1	3.1
Currently farming														
Yes	22	79	519	80	0.9	0.3	3.0	106	76	435	81	0.8	0.5	1.3
No	6	21	127	20				34	24	99	19			
Crops grown														
Field corn	25	89	504	78	3.1	0.9	11	104	74	425	79	0.9	0.5	1.4
Soybeans	25	89	490	76	3.0	0.9	10	102	72	413	77	0.9	0.6	1.3
Hay and alfalfa	15	54	314	48	1.5	0.6	3.6	68	48	261	49	1.3	0.8	2.0
Other grains	19	68	336	52	2.1	0.9	4.7	82	58	273	51	1.4	1.0	2.1
Tobacco	11	39	139	21	3.6	1.1	11	38	27	112	21	1.2	0.7	1.9
Cotton	5	18	65	10	2.0	0.6	5.9	11	8	59	11	0.5	0.3	1.1
Peanuts	5	18	64	10	2.0	0.7	6.1	14	10	55	10	0.8	0.4	1.5
Row crops	5	18	86	13	1.4	0.5	3.8	21	15	70	13	1.0	0.6	1.8
Orchard fruits	4	14	15	2	6.9	2.1	23	9	6	10	2	3.4	1.4	8.7
Silage	9	32	153	24	2.0	0.7	6.1	34	24	128	24	1.3	0.8	2.2
Animals raised														
Any animals	22	79	415	64	2.6	1.0	6.8	92	65	345	64	1.3	0.8	2.0
Cattle (dairy and beef)	19	68	308	47	2.8	1.2	6.7	72	51	255	48	1.4	0.9	2.1
Swine	8	29	216	33	0.8	0.3	2.2	38	27	186	35	0.8	0.5	1.3
Poultry	4	14	55	8	1.8	0.6	5.3	15	11	44	8	1.3	0.7	2.3

Orchard fruits are apples and peaches. Row crops are blueberries, cabbage, grapes, cucumbers, peppers, snap beans, strawberries, tomatoes, melons, and pumpkins.
*Defined as total IgE level of 100 kU/L or more.
†Adjusted for state.

low prevalence in the cohort (<3%).[2] Participants in the substudy were similar to the cohort as a whole with regard to farm history (Hoppin et al, 2012, unpublished data). The study was approved by institutional review boards of the University of Iowa, the National Institutes of Health, and its contractors.

Serum samples were analyzed for total immunoglobulin E and screened for fungal positivity to a fungal-mix (mx2) by fluoroenzymeimmunoassay using ImmunoCAP 100 (Phadia AB, Uppsala, Sweden). Sensitization to other allergens was not measured. If sera were fungal-mix positive, they were tested for specific IgE to 10 fungal species (*Aspergillus fumigatus, Penicillium chrysogenum, Alternaria alternata, Curvularia lunata, Epicoccum purpurascens, Phoma betae, Candida albicans, Botrytis cinerea, Fusarium proliferatum*, and *Cladosporium herbarum*). Fungal sensitization was defined as specific IgE levels of 0.35 kU/L or more to at least 1 fungus; atopy was defined as total IgE level of 100 kU/L or more.[3]

Pesticide applicators completed 3 questionnaires about their agricultural and medical history: one at enrollment and two 5-year follow-up interviews. Farming practices included specific crops and animals raised, growing up on a farm, and years worked or lived on a farm. Data from all 3 questionnaires were used for the analysis. Exact logistic regression models were used to estimate associations with farming characteristics and fungal sensitization or atopy while controlling for state; other factors such as age and smoking did not confound the associations.

The prevalence of fungal sensitization was 4%, and the prevalence of atopy was 21%. While the prevalence of atopy was greater for North Carolina farmers than for Iowa farmers (odds ratio [OR], 1.5; 95% CI, 1.0-2.1), fungal sensitization did not differ. Twenty-five percent of fungal-sensitized participants were not classified as atopic (n = 7; see Table E1 in this article's Online Repository at www.jacionline.org). Fungal-sensitized farmers (n = 28) were slightly older than those who were not (63 years vs 61 years); however, there was no difference in the average age of the 2 groups ($P = .27$).

Specific commodities were related to fungal sensitization (Table I). After adjusting for state, farmers who grew tobacco, orchard fruit, or raised animals, particularly cattle, were more likely to be fungal sensitized than farmers not performing these activities. Growing soybeans, field corn, or other grains was also positively associated with fungal sensitization but was not statistically significant. We lacked the ability to assess multiple crops in the same model because of the low prevalence of fungal sensitization and the high degree of correlation among crops. Sensitization to any specific fungal antigen was low, ~2% for all farmers and between 4% and 8% when limited to those classified as atopic (Table II). Sensitization to specific fungi showed that 11 of the 28 individuals (39%) were monosensitized and 17 (61%) were multisensitized.

Fungal sensitization was less common among these farmers than among white men 40 years and older from the National Health and Nutrition Examination Survey in 2005-2006.[4] Among farmers, sensitization prevalence to both *Alternaria* and *Aspergillus* was 2% (95% CI, 1%-3%), which was lower than that in the National Health and Nutrition Examination Survey, with 7% for *A alternata* (95% CI, 5%-9%) and 6% for *A fumigatus* (95% CI, 4%-7%). Atopy prevalence was also lower

TABLE II. Prevalence of fungal sensitization to 10 fungal agents among 677 male AHS farmers, 2006-2008

	All farmers (n = 677)		Atopic* farmers (n = 141)	
	n	Percent	n	Percent
Any fungal sensitization†	28	4	21	15
Eurotiales				
Aspergillus fumigatus	14	2	11	8
Penicillium chrysogenum	14	2	11	8
Pleosporales				
Alternaria alternata	14	2	8	6
Curvularia lunata	7	1	7	5
Epicoccum purpurascens	14	2	11	8
Phoma betae	14	2	11	8
Saccharomycetales				
Candida albicans	14	2	10	7
Helotiales				
Botrytis cinerea	7	1	6	4
Hypocreales				
Fusarium proliferatum	7	1	6	4
Capnodiales				
Cladosporium herbarum	7	1	6	4

*Defined as total IgE level of 100 kU/L or more.
†Sensitized (specific IgE level of ≥0.35kU/L) to any fungi.

(21%; 95% CI, 17%-25%) than that among the National Health and Nutrition Examination Survey participants (30%; 95% CI, 27%-33%).

Other studies of agricultural workers suggest greater fungal sensitization among farmers.[5-8] While no studies appear to have focused on the fungal sensitization associated with specific field crops, 18% of greenhouse workers were sensitized to at least 1 of 4 fungal species as measured by the skin prick test, much higher than what we observed even though our panel included the same fungi in addition to 6 other species.[5]

While our study is among the largest to characterize fungal sensitization among farmers, our analysis was limited because of the low prevalence of sensitization and the high prevalence of certain types of crop production and high correlation among these crops. Our panel contained 10 common fungal species; many, but not all, have been associated with commodity crops. If the panel included a greater diversity of species, the prevalence of sensitization may have been higher; however, given that most farmers were multisensitized to fungi, it is unlikely that the addition of other fungi would greatly alter our results. The participants were older farmers, and so a healthy worker effect is possible where sensitized farmers have been selected out of agriculture prior to sampling. Our population was not selected on the basis of respiratory disease history; thus, our lower estimates may reflect the fact that other studies may have been enriched for individuals with respiratory outcomes. The agricultural exposure history was well characterized by detailed questionnaires; however, no environmental exposure assessments were conducted. Because of the high degree of correlation between some crops, most notably corn and soybeans, and the high prevalence of growing these 2 crops, we lacked statistical power to fully evaluate whether one or both of these major crops contributed to fungal sensitization.

In conclusion, this study represents one of the largest and most heterogeneous studies of fungal sensitization with regard to agricultural activities. Both fungal sensitization and atopy were low in this sample of US farmers compared with the US population. Individuals working with tobacco, orchard fruit, or animals had a higher prevalence of fungal sensitization; however, the sample size limited our ability to evaluate the influence of specific crops. Because fungal exposures differ around the world and rates of allergen sensitization differ on the basis of farming history, future studies will need to focus not only on commodities produced and specific fungal agents but also on population characteristics that may have an impact on sensitization.

We thank Stuart Long for his assistance with data analysis. Findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Association of Schools of Public Health and/or the Centers for Disease Control and Prevention. Copies of questionnaires are available at www.aghealth.org/questionnaires.html. We used the P1REL201005, P3REL1090100, and AHSREL201004 releases of the AHS data set.

Stacy M. Endres, MPH[a,b]
Brett J. Green, PhD[c]
Paul K. Henneberger, ScD[b]
Dori R. Germolec, PhD[d]
Toni A. Bledsoe, PhD[c]
Donald H. Beezhold, PhD[c]
Stephanie J. London, MD[a]
Michael C. Alavanja, PhD[d]
Laura E. Beane Freeman, PhD[e]
Jane A. Hoppin, ScD[a]

From [a]the Epidemiology Branch and [d]the Toxicology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC; [b]the Division of Respiratory Disease Studies and [c]the Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WVa; and [e]the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Rockville, Md. E-mail: hoppin1@niehs.nih.gov.

This work was supported in part by the Association of Schools of Public Health and the Centers for Disease Control and Prevention fellowship program, the intramural research program of the National Institutes of Health, the National Institute of Environmental Health Sciences (Z01-ES049030), and the National Cancer Institute (Z01-CP010119) and an Interagency Agreement (Y1-ES-0001) between the National Institute for Occupational Safety and Health and the National Institute of Environmental Health Sciences.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Eduard W. Fungal spores: a critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. Crit Rev Toxicol 2009; 39:799-864.
2. Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, et al. Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the Agricultural Health Study. Neurotoxicol Teratol 2012;34:168-76.
3. Burney P, Malmberg E, Chinn S, Jarvis D, Luczynska C, Lai E. The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. J Allergy Clin Immunol 1997;99:314-22.
4. National Center for Health Statistics, US Centers for Disease Control and Prevention. Department of Health and Human Services (CHHS)/National Health and Nutrition Examination Survey (NHANES), 2005-2006. 2006. Available from: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm. Accessed June 1, 2011.
5. Monso E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J. Occupational asthma in greenhouse flower and ornamental plant growers. Am J Respir Crit Care Med 2002;165:954-60.
6. Rimac D, Macan J, Varnai VM, Vucemilo M, Matkovic K, Prester L, et al. Exposure to poultry dust and health effects in poultry workers: impact of mould and mite allergens. Int Arch Occup Environ Health 2010;83:9-19.
7. Terho EO, Vohlonen I, Husman K, Rautalahti M, Tukiainen H, Viander M. Sensitization to storage mites and other work-related and common allergens among Finnish dairy farmers. Eur J Respir Dis Suppl 1987;152:165-74.

8. Zhang Y, Chen J, Chen Y, Dong J, Wei Q, Lou J. Environmental mycological study and allergic respiratory disease among tobacco processing workers. J Occup Health 2005;47:181-7.

Available online May 26, 2012.
doi:10.1016/j.jaci.2012.04.018

Helminth infection is associated with decreased basophil responsiveness in human beings

To the Editor:

Helminth infections and allergic diseases are characterized by increases in IgE and type 2 cytokines such as IL-4. Despite their common immunopathogenesis, epidemiologic studies reveal an inverse relationship between the prevalence rates of these diseases[1,2] and a number of animal experiments have demonstrated that helminth infections can actively protect against the development of allergy.[2] While several mechanisms have been proposed, the pathophysiology underlying this phenomenon remains unclear.[2]

Recently, we demonstrated that chronic helminth infections suppress basophil responsiveness to IgE-mediated activation in mice.[3] This phenomenon may be a principal mechanism underlying helminth-mediated protection against allergy because basophils are increasingly being recognized as functionally important in allergic diseases. Basophils participate in the effector phase of allergic responses by releasing acute inflammatory mediators such as histamine after IgE-mediated activation. Through the release of IL-4, they also play a prominent role amplifying the type 2 responses that drive allergic diseases.[4,5] Given the many differences between murine and human basophils,[5,6] in this study we sought to determine whether basophil suppression occurs in human beings infected with helminths. To evaluate this, we compared basophil histamine release in helminth-infected children before and after anthelmintic treatment. Studies were approved by the institutional review boards at the Universidad San Francisco de Quito, Ecuador, and at the Uniformed Services University.

Parasitologic examinations were conducted on stool samples from 28 children aged 8 to 14 years in a rural community in Esmeraldas province, Ecuador. *Ascaris lumbricoides* and *Trichuris trichiura* eggs were found in the stool of all children, and *Hymenolepis nana* eggs were found in the stool of 2 children. Blood from children was collected in heparinized tubes and centrifuged. After centrifugation, plasma was carefully removed and blood cells were washed twice with PBS. Blood cells were resuspended to the original volume by using PBS, diluted with Histamine Release Buffer (Beckman Coulter, Inc, Indianapolis, Ind), and stimulated for 30 minutes with seven 4-fold concentrations of anti-IgE (0.0005–2 μg/mL, Sigma-Aldrich, St Louis, Mo) and ionomycin (5 μg/mL, Calbiochem, San Diego, Calif). Stimulated blood was centrifuged for 10 minutes at 400*g*, supernatant was acylated, and histamine levels determined by using a competitive histamine ELISA (Beckman Coulter, Inc). The percentage of total histamine released was calculated by dividing the amount of histamine released into the supernatant by the amount of histamine in a lysed aliquot of blood. Infected children were then treated orally with 3 daily 800 mg doses of albendazole and a single dose of ivermectin at 0.2 mg/kg. Two weeks after therapy, histamine release from blood basophils was measured again from 22 of the treated children by using identical stimulation conditions. To enable paired comparisons, samples only from the 22 children who provided blood before and after treatment were utilized in analyses. The 2-week time point was chosen because human basophil lifespan is estimated to be 2 to 14 days.[7] Efforts were made to standardize blood processing, and basophil activation studies were conducted on average 4.5 hours after blood draw.

When sufficient plasma was available, samples were analyzed for circulating total IgE levels by using a human IgE ELISA kit (Immunology Consultants Laboratory, Inc, Portland, Ore). Two-tailed Wilcoxon signed ranked test was used to determine statistical significance between paired samples. GraphPad Prism version 4.03 was used for all statistical analyses.

To evaluate whether helminth infections play a role in suppressing basophil function, histamine release from basophils of infected children was measured 2 weeks after anthelmintic therapy. As seen in Fig 1, substantial increases in basophil activation in response to IgE-mediated activation developed in endemic children after their infections were treated. Two weeks

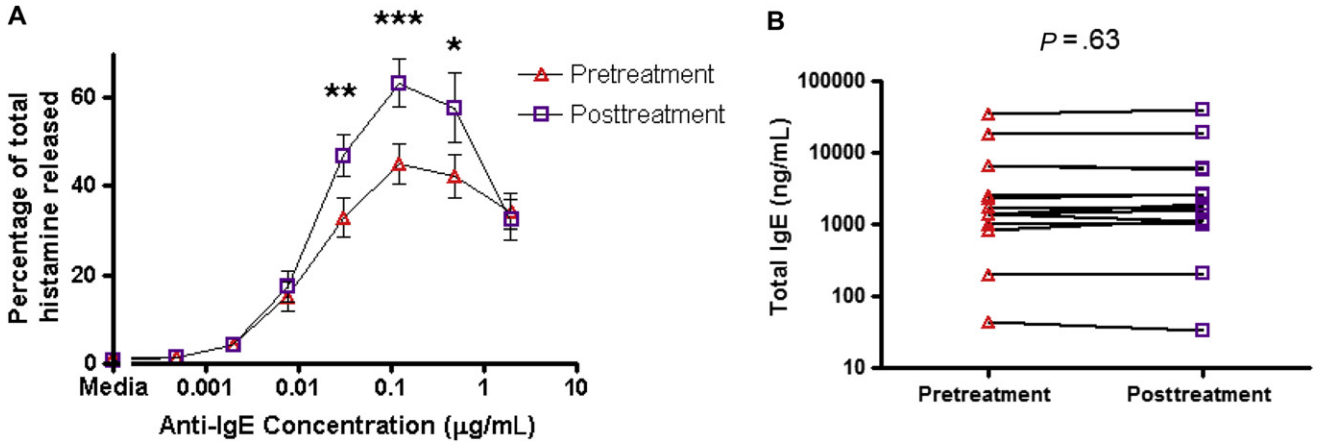

FIG 1. Blood basophil histamine release in response to anti-IgE and IgE levels from infected children before and 2 weeks after anthelmintic treatment. **A,** Histamine released after stimulation with increasing concentrations of anti-IgE (n = 22 for pre- and posttreatment groups; **P* < .05; ***P* < .01; ****P* < .001). **B,** Total IgE levels before and after treatment (n = 14 for pre- and posttreatment groups).

TABLE E1. Demographic, lifestyle, and medical characteristics of the 677 AHS farmers by fungal sensitization and atopic status

Characteristics	Fungal sensitized							
	Yes (n = 28)		No (n = 649)		Atopic (n = 141)*		Nonatopic (n = 536)	
Age (y) at blood draw, mean (SD)	63 ± 14		61 ± 12		63 ± 12		60 ± 12	
	n	%	n	%	n	%	n	%
Atopy*	21	75	120	18	141	100	0	0
Body mass index (kg/m^2)								
<25	6	21	122	19	26	19	102	19
25-30	12	43	327	50	65	46	274	51
>30	10	36	200	31	50	36	160	30
Smoking history								
Ever smoked	11	39	277	43	61	44	227	42
Never smoked	17	61	371	57	80	57	308	57
Alcohol consumption								
Ever	16	57	406	63	87	62	335	63
Never	12	43	243	37	54	38	201	38
State								
Iowa	13	46	329	51	61	43	281	52
North Carolina	15	54	320	49	80	57	255	48
Respiratory symptoms and diseases								
Hay fever	13	46	119	18	37	27	95	18
Wheeze in the past 12 mo	12	43	131	20	42	30	101	19

*Defined as total IgE level of 100 kU/L or more.